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DNA barcoding compliments morphological identification in tomato fruit borer, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

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ABSTRACT: Among Heliothine group of moths *viz., Helicoverpa armigera* (Hübner), *Helicoverpa assulta* (Guenée), *Heliothis peltigera* (Denis & Schiffermüller) and *Helicoverpa rama* (Bhattacharjee and Gupta), *Helicoverpa armigera*, is the most serious pest infesting many economically important crops. Though, *Helicoverpa* infesting vegetables *viz*., tomato, okra, cowpea, amaranthus, cucurbits etc in Kerala, their identity at species level is lacking. Hence, the larvae collected from tomato growing area were reared, preserved and subjected to further studies. Morphological characters *viz*, setal arrangement on prothoracic segment and genitalia structure of both the male and female adult moths were examined. Further, DNA barcoding was carried out to reveal the identity at molecular level. Both morphological characters and DNA barcoding confirmed the identity of species as *Helicoverpa armigera*.

KEYWORDS: *Helicoverpa armigera,* setae, male and female genitalia, DNA barcoding

INTRODUCTION

Heliothine group of moths include some of the most damaging insect pests of agricultural crops throughout the world. *Helicoverpa armigera* (Hübner), *Helicoverpa assulta* (Guenée) and *Heliothis peltigera* (Denis and Schiffermüller) belong to this group have been recorded from India (Jadhav and Armes, 1996).

Earlier, *Helicoverpa rama distinguished* as a species distinct from within the commonly accepted *H. armigera* (Bhattacharjee and Gupta, 1972), but now it has been synonymyzed

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with *H. armigera*. *Helicoverpa armigera*, popularly known as cotton bollworm or tomato fruit borer or gram pod borer, is the most serious pest among the four species infesting economically important crops in India. It has been recorded on more than 181 plant species from 45 families (Manjunath *et al*., 1989) causing annual loss to the tune of Rs. 2000 crores (Ignacimuthu and Jayaraj, 2003).

In Kerala, the incidence of *H. armigera* was reported on tomato, okra, cowpea, bittergourd and amaranthus (Mathew *et al*., 1996; Levin, 2004; Levin *et al*., 2004). However, its infestation also seen in other vegetables *viz*., okra, cowpea, cucurbits, etc. Pheromone traps (Helilure) set up for monitoring the *Helicoverpa armigera* population in the tomato fields of Thrissur and Palakkad had not collected a single adult moth. Moreover, the existence of three species of Heliothine moths in India prompted us to reveal the identity of species of *Helicoverpa* infesting tomato in Kerala.

Identification of insects based on morphological characters is the core principle of classical insect taxonomy. The larva of *H. armigera* had been distinguished from other related species on the basis of setal arrangement on prothorasic segments (Amate *et al*., 1998). Whereas, both the male and female genitalia structures adult moths were well studied in species of Heliothine moths (Hardwick, 1965; Hardwick, 1970).

The phenotypic plasticity and genetic variability in the character employed for specimen recognition can lead to incorrect identification. Similarly, the morphological keys are often effective for a particular life stages or gender and the use of keys often demands high level of expertise otherwise leads to misdiagnoses. This limitation inherent in the morphology based identification system which can be overcome with the help of novel microgenomic method called DNA barcoding (Hebert *et al*., 2003). This technique involves sequencing a short fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene, the 'DNA barcode,' from a taxonomically unknown specimen and performing comparisons with a reference library of barcodes of known species origin to establish a species-level identification (Wilson, 2012).

In the present study, the morphological characters *viz*., setal arrangement (chaetotaxy) on prothoracic segment in larva and genitalia structures of adult moths were examined. Further, DNA barcoding based on mitochondrial Cytochrome Oxidase1 (mtCO1) gene was carried out to confirm the identity of *Helicoverpa* species infesting tomato.

MATERIALS AND METHODS

Field collection of larva and maintenance of culture

Helicoverpa larvae collected from tomato growing areas of Palakkad and Thrissur were brought into laboratory along with fruits in plastic boxes containing six cavities and each cavity with dimension of 6.5 cm X 6.0 cm X 3.5 cm. Larvae were fed with plant parts for two days and later transferred them into multicavity trays and fed with chickpea based semi synthetic diet (Armes *et al.*, 1992). First to third instar larvae were fed with semi synthetic diet, whereas 4th instar

onwards soaked chickpea seeds were given to them and allowed for pupation. The pupae were sexed and kept in new plastic boxes for adult emergence.

1. Morphological characters

Setal arrangement on prothorax

Ten fifth instar larvae from above culture were selected and immobilized them by exposing to ethyl acetate (100%). The killed larvae were then transferred to glass vials containing ethyl alcohol (95%) to preserve the same for long period. The preserved specimen was transferred onto a glass slide and the position of setae on prothoracic segment was observed through stereo binocular microscope (Labomed®). The image of setae on prothoracic segment was captured using microscope with image analyser software (Leica®) and compared with setal map and diagnostic key of *Helicoverpa armigera* developed by Amate *et al*. (1998).

Adult genitalia structures

Both the male and female adult moths were selected and killed them by exposing to ethyl acetate (100%). The abdomen of adult male moth of *Helicoverpa* was detached with blunt forceps and dropped in ethyl alcohol (95%) for one minute and transferred into a labelled test tube containing 10 per cent potassium hydroxide (KOH). The test tube was kept over spirit lamp for 10-15 minutes and the boiled specimen was allowed to cool for 10-20 minutes. The softened abdomen was placed in a Petri dish containing distilled water to remove KOH.

The soft abdominal skin was cleared out with stainless steel needles. Base of the abdomen was held with straight forceps and pressed gently with round end of curve tipped forceps from base to apex and extruded the entire genitalia. This process was carried out carefully without damaging the aedeagus. The genitalia was placed on a glass slide with drops of water and the genital valves were stretched out with the help of needles, the *harpes* were opened using micro pins to obtain a full face view of inner structures. The genitalia were stained with acid fuschin for two minutes and the excess stain was removed. Drops of Canada balsm were added above the genitalia and xyline was used to remove the air bubble trapped inside the mountant. Cover slip was placed on the slide gently and the glass slide was allowed to dry for one to two days at room temperature, sealed the sides of cover slip with nail polish. The prepared glass slide of genitalia was used for microscopic study.

Compared to the dissection of male genitalia, female genitalia required a different approach in order to have the internal parts exposed. After initial procedures as that of male genitalia, female abdominal skin between 6^{th} and 7^{th} segments was removed with needles; utmost care was taken not to tear the bursae. The extruded female genitalia was then placed on a glass slide with little water, subsequently stained and slide prepared similar to that of male genitalia for comparison.

2. DNA barcoding of tomato fruit borer

Isolation of genomic DNA from tomato fruit borer larva and quality checking

The total genomic DNA of tomato fruit borer was isolated using modified CTAB method. Briefly, a single larva was ground in pre chilled mortar and pestle with the addition of 500 µl pre-warmed modified CTAB extraction buffer (2%) [CTAB (2%) 100 mM Tris-HCl [pH-8], 10 mM EDTA (pH-8), 1.5 M NaCl ,2-β mercaptoethanol (2 %)]. The homogenised sample was transferred into an autoclaved 1.5 ml Eppendorf tube. The contents were mixed well and incubated at 65^0C for 1 h with occasional mixing by gentle inversion. An equal volume of chloroform: isoamyl alcohol (24:1) was added and centrifuged at 6000 rpm for 15 min. at 4^0C. The supernatant was collected in a fresh 1.5 ml Eppendorf tube, added 40 µl of sodium acetate (3M) and 600 µl of ethanol (95%), incubated in a deep freezer at -20^0C for 20 min. and centrifuged at 8000 rpm for 10 min. at 4^0C and the genomic DNA pellet was precipitated out. The DNA pellet was washed with ethanol (70 %) by centrifugation at 8000rpm for 10 min. The DNA pellet was air dried for 15 min, dissolved in 25 µl of autoclaved distilled water and stored in deep freezer (-80^0C) for future use. The quality of isolated DNA was assessed by 1 per cent agarose electrophoresis.

Polymerase Chain Reaction (PCR) with DNA barcode primer and sequencing

Good quality genomic DNA (50 ng/µl) isolated from tomato fruit borer larva was used for DNA barcoding. The universal barcode primer [Hebert *et al*. (2003)] specific to mitochondrial cytochrome oxidase I (mtCO1) was used for PCR amplification. The mtCO1 region was amplified by polymerase chain reaction from genomic DNA using the universal barcode primers (**F**: HCO - 5TAAACTTCAGGGTGACCAAAAAATCA -3', **R:** LCO - 5' - GGTCAACAAATCATAAAGATATTGG -3) in Veriti Thermal Cycler (Applied Biosystems®). The PCR reaction was performed using 5 µl template DNA (50 ng), 0.5 µl of the forward and reverse primers, 0.5 µl of 10 mM dNTP (Genei®), 0.2 µl of Taq DNA polymerase (Genei®), 2.5 µl of Taq DNA buffer B(Genei®), 0.7 µl of $MgCl_2$ and 14.5 µl of Millipore® water. The PCR conditions were programmed as, Lid temperature 98^0C, initial denaturation 94^0C for 5 min, 40 cycles each of denaturation 94^0C for 45 seconds, primer annealing 55^0C for 45 sec and primer extension 72^0C for 45 sec, followed by 10 min extension at 72^0C and storage at 4^0C. The amplified PCR product was run on agarose (1%) gel electrophoresis and the product was sequenced at SciGenom labs, Cochin. Fifty larvae collected from tomato fields of Thrissur and Palakkad and five representative samples were sent for sequencing.

Sequence analysis and submission to GenBank, NCBI and Barcode of Life Database (BOLD)

The sequence generated from this study was analyzed for sequence homology using the nucleotide BLAST at NCBI, submitted to BankIt, GenBank and the accession numbers were generated. Further the specimen details and sequences were submitted to BOLD database and barcode for *H. armigera* was generated.

RESULTS

Morphological characters

Prothoracic setal arrangement of fifth instar larvae was observed and the image was captured using microscope with image analyser software. A well developed prothoracic shield was present in larva and altogether 11 primary setae were observed on prothorax *viz*., two dorsal seta (D_1 and D_2), two additional seta (XD_1 and XD_2), two subdorsal seta (SD_1 and SD_2), two lateral seta (L_1 and L_2), two subventral seta (SV_1 and SV_2) and one ventral seta (V_1). Both the additional seate XD_1 and XD_2 lied near to the anterior margin of prothoracic shield. XD_1 was situated near the mid longitudinal line of the half of shield, while XD_2 near the lateral margin. XD_2 was slightly shorter than XD_1. The dorsal seta D_1 situated posterodorsad to XD_1whereas D_2 posterolaterad to XD_2. The sub dorsal setae SD_1 and SD_2 lied near to lateral margin of prothoracic shield. Two lateral setae L_1 and L_2 lied anterior and horizontally aligned to spiracles, among them L_1 lied laterad to SD_1 and L_2 was identical to SD_2. Subventral setae SV_1 and SV_2 lied above coxa and SV_1 situated anterior to SV_2. The ventral group consisted of a single seta V_1which was situated post coxal and most ventral in position (Fig. 1).

The male genitalia were dissected out from the adult moths and slides were prepared. The parts of male genitalia of adult moth observed were uncus-a hook like structure with hairs arise from caudal end of tegumen, socci- paired organs arising from base of the uncus above gnathos, gnathos-paired organs arise from base of uncus and normally fused at tip into a strong hook, saccus- cephalic portion of vinculum, valves- clasping organs, corona-scleotized spines (Fig. 2). In male genitalia, uncus moderately long, well developed, simple, cylindrical, hook like with narrow towards tip; tegumen inverted U shaped; vinculum V shaped, valve long, apically broadened with no projection; corona numerous closely set seta arranged in several rows; saccus short, stouter with curved apical portion. To examine the aedeagus, the valves were removed first by holding the aedeagus at the base with one set of forceps and pulled both the valves together with the other forceps, thus the valves from the aedeagus were detached. The aedeagus was elongated, simple, cylindrical, weakly sclerotised structure. The numbers of cornuti (sclerotized spine) inside the aedeagus were 12 (Fig. 3a). The long spiral tube occasionally armed with spine called vesica was extended out from the aedeagus (Fig. 3b).

The various parts of female genitalia observed were ovipositor, a flattend sclerotized hairy lobe; anterior and posterior apophysis, setae like; ductus burase, duct connecting to bursa copulatix wherein sperms from male deposited during copulation. The ovipositor lobes were well developed, setosed, anterior and posterior apophysis almost of same length, ductus bursae sclerotized towards papilla analis, corpus bursae is oval shaped with 3 signum (Fig. 4).

DNA barcoding of tomato fruit borer

The mtCO1 region was amplified with universal DNA bacode primer in a thermal cycler and the

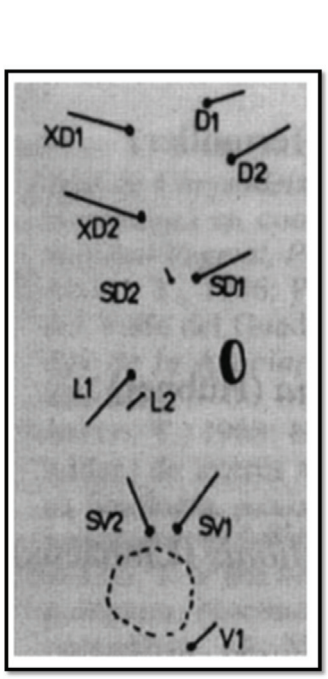
PCR product gave an intact band at 700 bp when resolved at 1 per cent agarose gel (Fig. 5). The sequence generated from tomato fruit borer consisted of 608 bp and homology of sequence with other reported sequences were analysed. The sequence showed significant homology to *H.armigera* mitochondrial Cytochrome Oxidase CO1 gene already deposited in the public domain database using 'blast n' search tool. The blast results showed 100 per cent query coverage and 99 per cent identity to *H. armigera* mtCO1gene. Then the sequence was aligned and annotated using bioinformatics tools, BioEdit and MEGA6. The sequences thus obtained were submitted to BankIt, NCBI under the accession number KM403206 and KP210095.

An account was opened in workbench session of BOLD systems v3 database and a new project 'RMTKL'was created. Specimen data *viz*., specimen identifiers, specimen taxonomy, specimen details, collection details was submitted and an auto generated process ID 'RMTKL001-14' was obtained. Further, primer details, high resolution specimen images, mitochondrial DNA sequences (fasta) and the trace files (.ab1) obtained from sequencer were uploaded to the database and the corresponding barcode of *H. armigera* (Fig. 6) was generated. Upon verification of DNA sequences submitted, the database allotted barcode index number (BIN), BOLD: AAA5223. Altogether 560 sequences of *H. armigera* were coming under the allotted BIN. Based on the distance model kimura 2 parameter, a BOLD taxon ID tree (Fig. 7) was constructed in database. It showed that the nearest neighbourhood of our sequence was *H. armigera* (Unpublished ID) sequence deposited from India. However, the sequences of *H. armigera* deposited in database from Punjab, Maharashtra, South Africa, United Kingdom, Kenya, Pakistan, Brazil, Italy and Germany also shared similarity with our sequences.

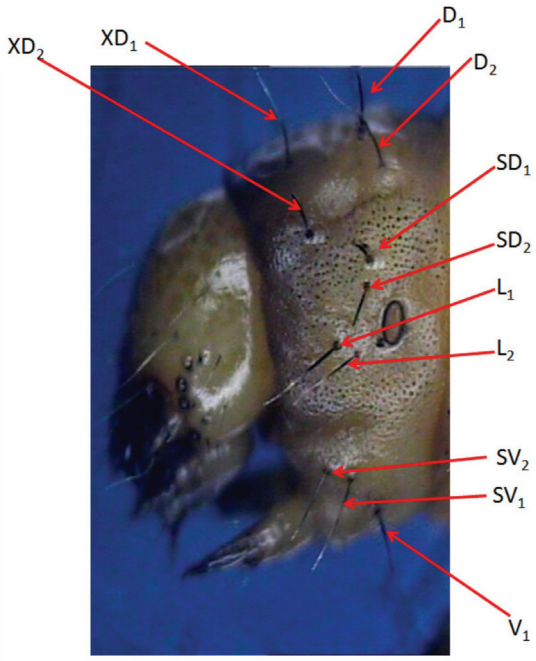
DISCUSSION

Morphological characters

The identification of pests in larval stage is difficult when compared to the adult stage, hence formal keys for the identification of caterpillar heavily depends on chaetotaxy, particularly primary setae (Waganer, 2005). The basic number of primary setae on body segment was of lepidopteran larva was11 and had a specific name based on position, it included two dorsal, two subdorsal, three lateral, three sub ventral and one ventral, and sometimes the prothorax bear two additional setae also (Stehr, 1987). However, in the present study altogether 11 primary setae were observed on prothoracic segments, it included two each of dorsal, subdorsal, lateral, subventral and additional setae, one ventral seta and the lateral setae aligned horizontally with prothoracic spiracle. This is in accordance with Amate *et al*. (1998) who prepared diagnostic key based on above characters to distinguish *H. armigera* from related species. The position of each seta on prothoracic segment was recorded and that was in consonance with Goel (2003) who described the taxonomy of Noctuidae with special reference to immature stages. Whereas, according to Sri *et al*. (1998) *H. armigera* larve were greenish in colour with dark coloured longitudinal stripes and had a dark prothoracic shield extended up to the margin of subdorsal seta (SD_1).

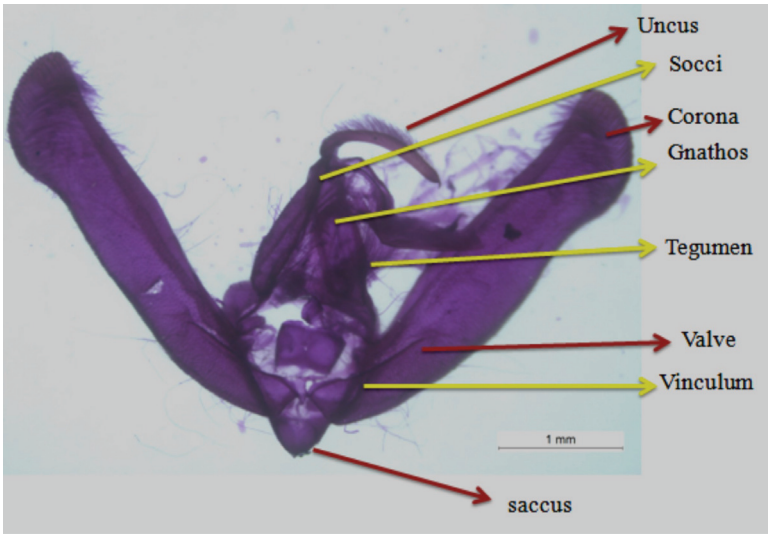


(Setal map, Amate *et al*., 1998)



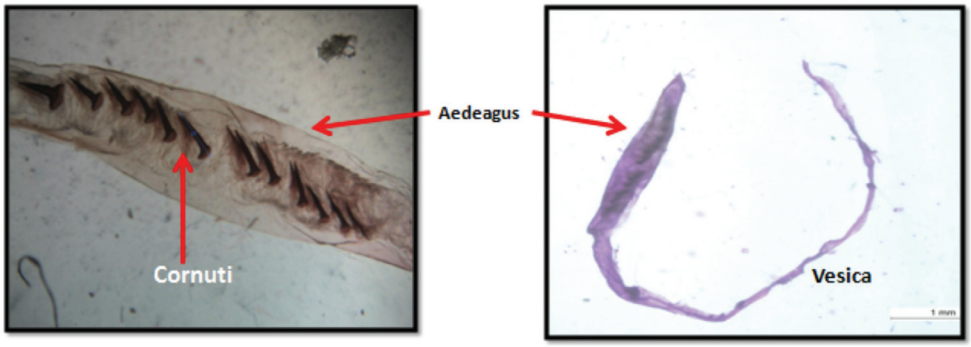
(Magnification 25x)

Fig.1 Setal arrangement on prothorax



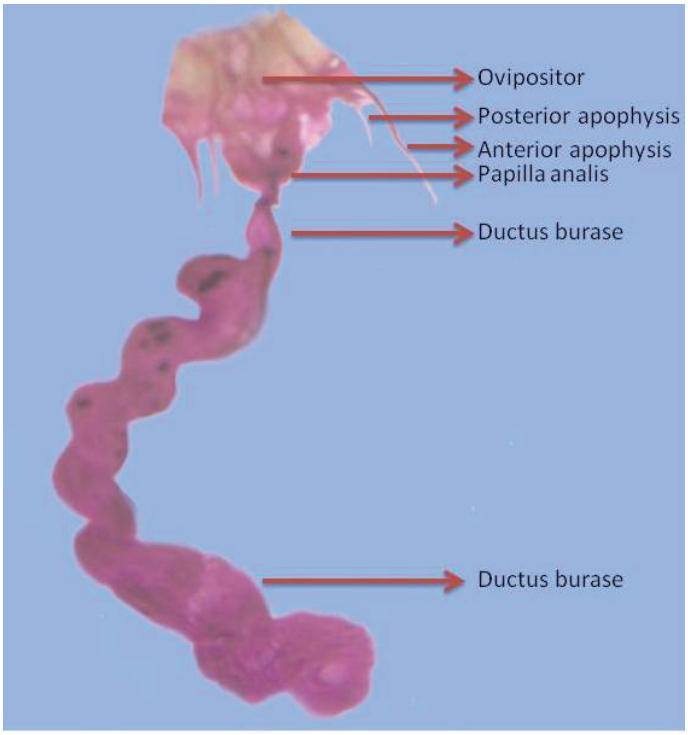
(Magnification 25x)

Fig 2. Male genitalia of *Helicoverpa armigera*



(Dissected male genitalia - magnification 100x) (Dissected male genitalia - magnification 25x)

Fig 3a. Aedeagus and Cornuti **Fig 3b. Aedeagus and Vesica**



(Magnification 25x)

Fig. 4 Female genitalia of *Helicoverpa armigera*

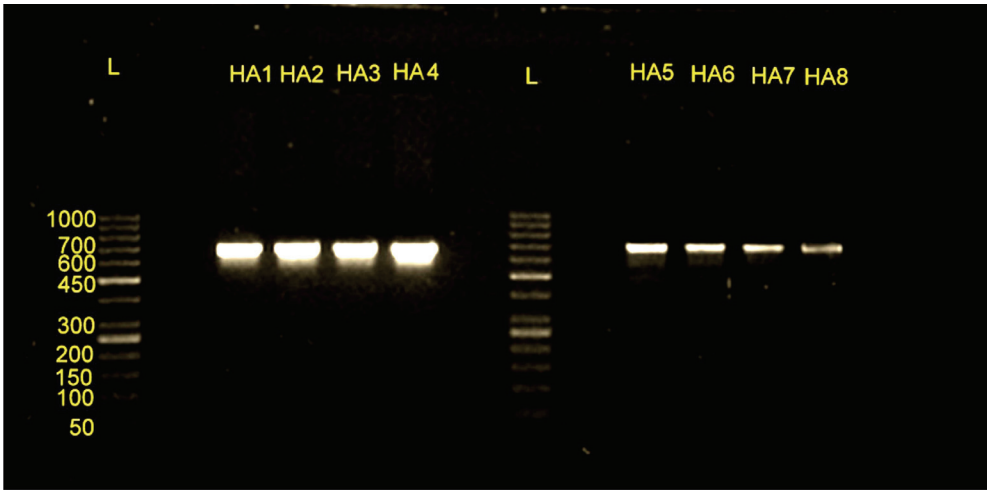


Fig. 5 Agarose gel electrophoresis of genomic DNA isolated from *Helicoverpa* upon amplification with mtCO1 primer. (L: 50 bp ladder, H1-H8: *Helicoverpa* DNA samples)

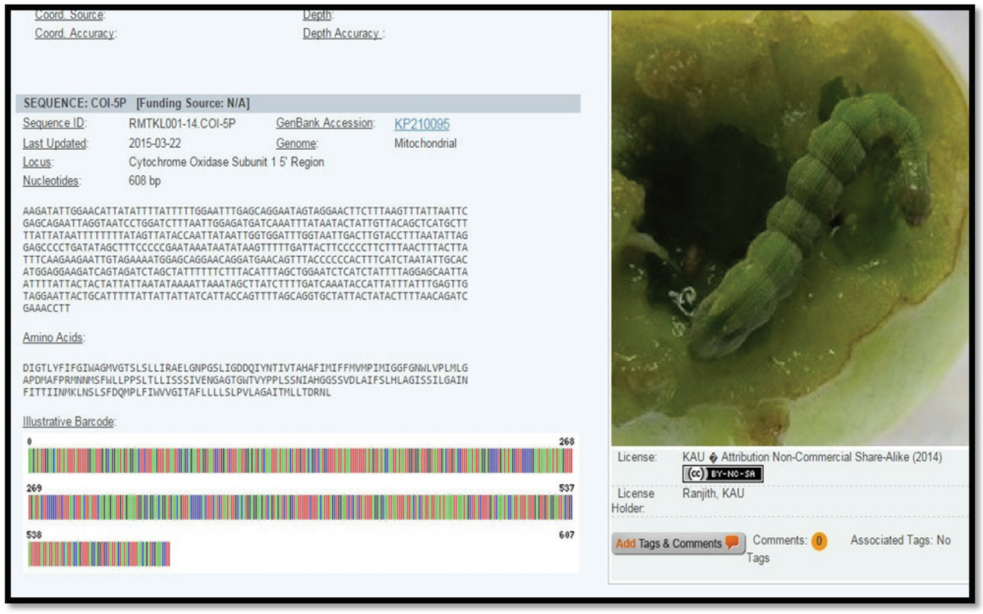


Fig. 6 DNA barcode of *H. armigera* generated by BOLD systems v3

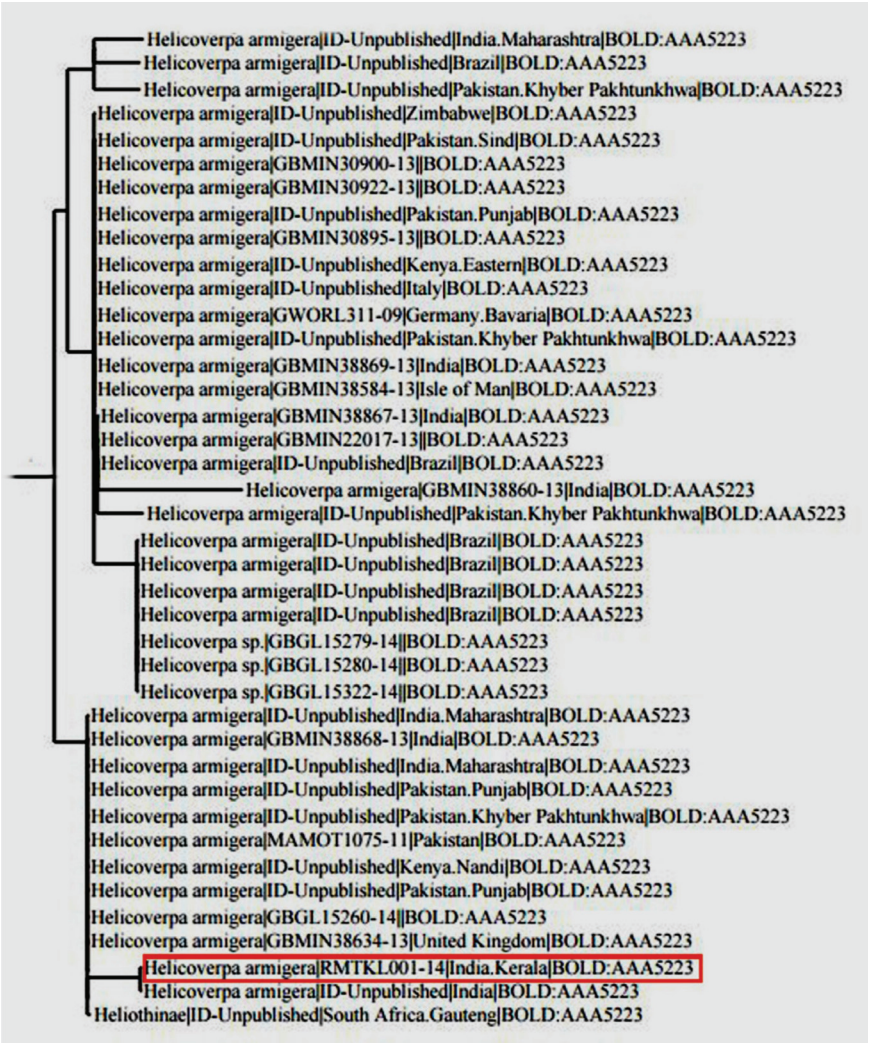


Fig. 7 A section of BOLD ID tree constructed in database

The consistency in genital character is universally recognised and as in any other taxonomic study, identification of species by means of genitalia depends upon a valid recognition of morphological characters (Siverly, 1947). The observation made on male genitalia structure was in accordance with previous workers. According to Hardwick (1970) male genitalia of *H. armigera* consisted of long to moderately long valves with broadened apical possed numerous coronas. Whereas, Brambila (2009) distinguished the species of *H. armigera* based on cornuti count. If the count of cornuti sets were equal to or less than 12, it could be *H. armigera*, and

if the count of cornuti sets exceeds more than 12 sets, it might probably be *H. zea*. If the aedeagus had no cornuti, or very few, the specimen was probably aberrant and sterile.

The adult female genitalia were dissected out and the various parts were recorded. The observation made are in agreement with Hardwick (1965), who reported that in female genitalia of *H. armigera*, dorsal sclerotization at the base of appendix bursae was restricted and it terminated apically in a normal dilation and lumen surface of appendix bursae clothed with spicules. Whereas, ductus bursae and appendix bursae in female in *H. armigera* were observed for possible variation in adult female moth infesting different host plants. Among the population collected from different crops, significantly the highest length of ductus bursae and appendix bursae was recorded in *H. armigera* population collected from chickpea (Patil *et al*., 2012).

DNA barcoding of tomato fruit borer

The barcode involves DNA sequence analysis of a portion (typically between 600- 900 bp) of the mitochondrial gene cytochrome c oxidase subunit I (COI). In the present study, we used the mtCO1 gene of *Helicoverpa* to reveal its species identity. The gene sequences were submitted to BOLD and corresponding barcode for *Helicoverpa armigera* was generated. Possibly we have been made the pioneer attempt to barcode *H. armigera* infesting tomato in Kerala.

We isolated DNA from larval stage of the insect and through barcoding techniques revealed the identity of specimen. The result envisaged the feasibility of using DNA barcode to rapidly assign the unknown specimen at different developmental stages either as a complement to morphological analysis or as the primary diagnostic indicator in cases where the requisite morphological keys are unavailable (Hebert *et al*., 2003).

The data base comparing the unknown barcode sequence using pairwise sequence divergence calculations (e.g., the Kimura 2-parameter model) as visualized using a neighbor-joining (NJ) tree. Based on the distance model kimura 2 parameter analysis, the nearest neighbourhood of our specimen was from India. The similar methodology was used effectively in a elucidating the cryptic aphid species in India (Rebijith *et al*, 2003).

Genetic diversity analysis of tomato fruit borer, *H. armigera* based on mitochondrial cytochrome oxidase-I (mtCO-I) showed that there was no significant variations in the CO-I sequences of *H. armigera* collected on various hosts and geographical locations. However, the phylogenetic tree constructed from the CO1 sequences indicated the possibility of emerging host associated genetic differences in *H. armigera* populations (Asokan *et al*., 2012).

DNA barcoding had been successfully applied in studying the lepidopteran specimens and correctly assigned them in taxonomic category. The barcode comparisons were successfully

applied to distinguish between closely related *Helicoverpa* species, *H. armigera* and *H. zea* (Behere *et al*. 2007). Studies indicated that the successful application of barcoding for species assignment might be taxa-dependent, but with poorly studied or recently diverging groups it became problematic. However, this method has potential for facilitating the identification of invasive insect pests (Floyd *et al*., 2010). DNA barcoding supplemented the morphological methods for identifying the invasive armyworm, *Spodoptera* species in Florida (Nagoshi *et al*., 2011).

In the present study the morphological characters *viz*., setal arrangement on prothorax of larva, genitalia structure of both male and female adult moths were explored and further DNA based identification system, DNA barcoding based on mitochondrial cytochrome oxidase 1 gene confirmed the species level of identity of tomato fruit borer as *H. armigera.* The identification of insects' pest based on both the morphological and molecular traits might be helpful in revealing the divergence of species, evolution of biotypes and species complex existing in the crop ecosystem, thus helps to evolve insect specific management strategies to reduce their menace in economically important crops.

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Selection of best performing *Apis cerana indica* Fab. colonies for stock improvement based on comparison of economic characters

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ABSTRACT: The comparative performance on economic characters of Indian bee *Apis cerana indica* Fab. collected from 18 locations of different beekeeping pockets in Kerala were studied during 2012-2013 to find out better performing colonies for selective breeding. The bee colonies collected from highland location Rajakkat (L3) and Rosemala (L6) were black morph and midland location Pathanapuram (L11) and lowland location Kadakkal (L18) were yellow morph and bees from other locations were common brown bees. The black and yellow morphs of *A. cerana indica* are being reported from Kerala for the first time. The black and yellow bee colonies recorded significantly higher mean bee strength, brood area, pollen storage area, honey storage area and honey yield compared to common brown bees. Both black and yellow bee morphs had more tolerance/ immunity against diseases compared to common bees. Absconding behavior was more in black bees while it was not recorded in yellow bees. These black and yellow bee colonies can be utilized for stock improvement through selective breeding for enhanced honey production.

KEY WORDS: *Apis cerana indica*, black morph, yellow morph, comparative performance, brood area, pollen storage area, honey yield

INTRODUCTION

The honey bee, *Apis cerana indica* is the predominant bee species widely used for commercial beekeeping in Kerala. Even though the industry had progressed recently, honey production is not upto the desired level due to lack of colonies /strains with desirable traits. Verma (1994)

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pointed out that a solution for low honey production, diseases, swarming and absconding characters of the species has to be worked out for sustainable apiculture with Indian bee. According to Chhuneja (2006 a,b), the only method for achieving the target will be stock improvement of the Indian bee *A. c. indica* by identifying the best genetic material from already available stocks, segregate promising colonies/queen bees on the basis of important economic and behavioural parameters and subsequently adopting inter-breeding through different mating combinations, avoiding any inbreeding.

Although beekeeping is a common practice in Kerala, studies on honey bee productivity or stock improvement of *A. cerana indica* is lacking. Hence an attempt was made to identify Indian bee colonies with better viable characters from among the existing bee colonies in Kerala.

MATERIALS AND METHODS

Eighteen locations from three natural topographic divisions of Kerala *viz.*, Highland (750 – 1700 m above MSL), Midland (100-750 m above MSL) and Lowland (25 – 100 m above MSL) (Table 1) were selected for the present study.

A. cerana indica colonies were selected from the apiaries of bee breeders / progressive beekeepers in locations mentioned in Table 1. Selection of colonies was done by checking colony registers maintained by the beekeepers with data on the performance and honey yield of the colonies in the previous years and also by visual observation of the colonies. Colonies with newly mated queen, six combs and approximately same bee strength (three frames) were selected. Three such colonies were collected from each location, marked and were brought to an apiary at Kadakkal, Kollam district. The colonies were kept strong and healthy, under same condition, by adopting the management practices recommended by the Kerala Agricultural University POP (Package of Practices).

Comparative performances on the economic characters (bee strength, brood area, pollen storage area, honey storage area and honey yield) were recorded from August 2012 to July 2013. Observations were recorded at 15 day intervals. Bee strength was assessed by counting comb well covered with bees on the two sides as one (Taha, 2007). The brood area, pollen storage area and honey storage area were recorded using a grid, which consist of a number of squares each measuring one square centimeter in area. The cells with brood / pollen/honey scattered in a comb was counted separately and converted into square cetimeters (Verma, 1998). Honey from the super chambers was extracted at intervals of eight days and weighed. Disease incidence was recorded through visual observation at fortnightly intervals. Number of colonies absconded were recorded at 15 day intervals. The data obtained were subjected to analysis of variance.

Table 1. Locations selected for collection of *Apis cerana indica* colonies

Topographical division	Agroclimatic zone	Location code	Location (District)	Altitude M) Above MSL	Longitude (°E)	Latitude (° N)
	Northern	L1	Sulthan Bethery (Wayanad)	1000	76°.2990	11°.72127
		L2	Panathady (Kasaragod)	750	75°.1302	12°.21327
Highland	Central	L3	Rajakat (Idukki)	1700	77°.0667	10°.1213
		L4	Adimali (Idukki)	1100	76°.9561	10°.0148
	Southern	L5	Amboori (Trivandum)	980	77°.8223	8°. 5025
		L6	Rosemala (Kollam)	1100	77°.1431	8°.9667
	Northern	L7	Peravoor (Kannur)	125	78.8256	9°.6729
		L8	Thamarassery (Kozhikode)	722	75°.3411	11°. 4912
Midland	Central	L9	Palakkad (Palakkad)	467	76°.3911	10°. 4625
		L10	Mundakayam (Kottayam)	330	76°.8833	9°.5500
	Southern	L11	Pathanapuram (Kollam)	120	76°.8882	9°.10 86
		L12	Nedumangadu (Trivandrum)	223	77°.0012	8°. 35 60
	Northern	L13	Ulikkal (Kannur)	25	75°.3900	12°.2031
		L14	Parappa (Kasaragod)	38	75°.2254	12°.3617
Lowland	Central	L15	Cheruthuruthi (Thrissur)	49	76°.2733	10°.7433
		L16	Perumbavoor (Ernakulam)	33	76°.4784	10°.1211
	Southern	L17	Neyyattinkara (Trivandrum)	75	77°.0833	8°.4240
		L18	Kadakkal (Kollam)	75	76°.9137	8°.8316

RESULTS

Comparative performance of *A. cerana indica* colonies from different locations

Two distinct colour morphs of *A. cerana indica* could be observed from certain beekeeping pockets in the present study, in addition to the common brown bees, Bees from L3 and L6 were black morphs, those from L11 and L18 were yellow morphs and bees from other locations were common brown bees. The colour of the queen and workers was differing compared to common brown bees. In black bees the abdomen of the queen was very black and that of yellow bees was yellowish brown and in common brown bees it was dark brown. The colour of the abdomen of worker bees were also very distinctive (Fig. 1,2,3).

a. Bee strength (No. of bee frames)

Data on bee strength of colonies from different locations are shown in Table 2. Mean bee

Table 2. Monthly variation in bee strength (No. of frames with bees on both sides) of *Apis cerana indica* colonies from different locations during 2012-13

Locations	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Pooled mean
L1	3.001	3.208	3.750	3.937	2.574	2.966	3.276	3.625	3.014	2.479	2.995	3.282	3.175
L2	3.155	3.250	4.500	5.841	3.791	4.608	5.227	5.052	4.255	3.887	4.451	5.034	4.421
L3	3.145	3.335	5.083	6.537	5.175	7.012	7.452	7.123	6.104	4.708	6.073	7.010	5.729
L4	3.130	3.166	3.916	5.379	3.495	4.449	4.885	4.010	3.645	3.470	4.383	4.911	4.069
L5	3.112	3.125	4.665	5.441	3.317	3.795	4.289	4.125	4.525	3.455	3.766	4.087	3.975
L6	3.150	3.375	5.249	6.525	3.474	4.912	5.624	5.220	4.350	5.782	6.011	6.622	5.025
L7	3.160	3.165	4.145	5.212	3.533	4.575	5.232	5.120	4.120	3.786	4.241	4.848	4.261
L8	3.255	3.541	3.958	4.204	2.646	3.195	3.625	3.401	3.247	2.680	2.953	3.353	3.338
L9	3.300	3.665	5.335	5.829	3.750	4.587	5.312	5.184	4.100	3.987	4.407	5.046	4.541
L10	3.205	3.335	4.224	4.424	2.533	3.416	3.872	3.630	3.533	2.765	3.120	3.556	3.467
L11	3.148	3.333	5.624	6.496	4.112	5.837	6.568	6.272	5.540	4.570	5.252	6.115	5.238
L12	3.205	3.291	4.200	4.025	2.391	3.020	3.414	3.648	3.224	2.640	2.851	3.115	3.252
L13	3.150	3.233	4.666	5.654	3.258	4.541	5.078	5.011	4.333	4.065	4.504	4.753	4.353
L14	3.175	3.183	4.835	5.516	3.766	4.837	5.405	5.200	5.000	4.250	4.620	4.999	4.566
L15	3.125	3.191	4.808	5.466	3.608	4.208	4.778	4.555	4.125	3.906	4.499	4.831	4.258
L16	3.175	3.275	4.635	5.570	3.687	4.624	5.067	5.012	4.720	3.945	4.163	4.411	4.357
L17	3.120	3.250	4.274	5.200	3.529	3.741	3.829	3.432	3.135	2.822	3.027	3.193	3.546
L18	3.165	3.358	5.333	6.133	4.308	5.658	6.911	6.244	5.344	4.780	5.339	5.996	5.214
CD	*NS*	*NS*	*0.5174*	*1.0948*	*1.657*	*2.551*	*2.8743*	*2.9641*	*2.5743*	*2.1045*	*2.5725*	*2.7324*	

Fig.1 Black morph of *Apis cerana indica*

Fig. 2 Yellow morph of *Apis cerana indica*

Fig.3 Common brown morph of *Apis cerana indica*

strength in colonies from all the locations was same during August and September. Highest mean bee strength (5.624) was observed in colonies from L11 followed by L9 (5.335) and L18 (5.333) during October which was on par with that of black bees from L6 (5.249) and L3 (5.083). Bees from L1 showed the least mean bee strength (3.750). There was a gradual increase in bee strength in September and reach a high level in November. Black bee colonies from L3 and L6 had mean bee strength of 6.537 and 6.525 respectively during November which were on par with those of yellow bee colonies from L11 and L18 (6.496 and 6.133 respectively) compared to common brown bees from other locations. During the month of December highest mean strength was observed in black bees from L3 (5.175) which was significantly higher than that of bee colonies from other locations.

Bee strength was highest (7.021) in black bee colonies from L3 followed by L11 (5.837) and L18 (5.658) during January and least was in brown bees from L1 (2.966).The second peak in bee strength was observed during February. Mean bee strength was highest in black bee colonies from L3 and L6 (7.452 and 5.624 respectively) and in yellow bees from L18 and L11 (6.911 and 6.568 respectively) which were on par and significantly higher than brown bees. Same trend was observed during March also. The black bees (L3, L6) and yellow bees (L11, L18) recorded highest mean bee strength (7.123, 5.220, 6.272, 6.244 respectively) which were on par with that of L2, L6,L7, L9, L13,L14,L15,L16. During April mean bee strength declined in all the colonies and black bees from L3 recorded the highest (6.104) which was on par with that of yellow bees from L11 and L18 (5.540 and 5.344 respectively). Bees from all other locations recorded significantly lower bee strength.

The mean strength decreased again during May and highest bee strength (5.782) was

observed in black bee colonies from L6 which was on par with that of yellow bees from L11 and black bees from L3, L6. During June also the black bees from L3 and L6 showed highest bee strength (6.073 and 6.011) which was on par with that of yellow bees from L18 and L11 (5.339, 5.252 respectively). Same trend was noticed during July also with highest bee strength of 7.010 in L3 followed by L6. Least mean bee strength was observed in common brown bees from L12.

b. Brood area (cm^2)

The black bees from L3 had the highest mean brood area (553.167 cm^2) during September (Table 3) which was significantly higher than other colonies and it was followed by black bees from L6 (438.833 cm^2), yellow bees from L18 (438.165 cm^2) and L11 (429.833 cm^2). The lowest brood area was recorded in common brown bees from L1 (233.335 cm^2). During October, highest mean brood area was recorded in black bees from L3 (912.665 cm^2) which was on par with that of black bees from L6 (830.165 cm^2) which were statistically higher to other locations. Mean brood area in yellow bee colonies from L18 (729.500 cm^2) and L11 (726.167 cm^2) were on par. Least brood development was observed in colonies from L1 (395.166 cm^2). The black bees from L3 recorded the highest mean brood area (1260.084 cm^2) during November which was on par with that of black bees from L6 (1165.000 cm^2). Mean brood area in yellow bee colonies from L11 (1035.751 $cm^{2)}$ was on par with that of bees from L6. Common bees from L12 showed the lowest brood area (562.000 cm^2) during this period. During December, maximum mean brood area of 879.417 cm^2 was recorded in black bee colonies from L3 which was on par with that of yellow bees from L18 (704.666 cm^2) and were statistically higher to bees of other locations. It was followed by the yellow bees from location 11 (630.085 cm^2), common bees from L4 (559.083 cm^2) and black bees from location 6 (539.250 cm^2). The common bees from location 10 showed the least brood development (281.750 cm^2).

Maximum mean brood area was recorded in black bee colonies from L3 during January (1438.417 cm^2) which was statistically significant from bees of other locations. It was followed by the yellow bees from L18 and 11which were on par (979.917 cm^2 and 841.667 cm^2 respectively). Least mean brood area of 335.250 cm^2 was recorded in common bees from L8. During February, also the black bees from L3 recorded highest mean brood area (1666.667 cm^2) which was on par with that of yellow bees from L18 (1240.250 cm^2) followed by bees from L11 and L6 which were also on par (1189.309 cm^2, 1134.122 cm^2). Lowest brood area was recorded in bees from location 8 (396.634 cm^2). All the colonies from other locations recorded comparatively less brood area. Highest mean brood area (1587.145 cm^2) was recorded in black bees from L3 during March which was significantly higher. Yellow bees from L18 recorded a mean brood area of 1186.867 cm^2 followed by bees from L11 and L6 (1098.167 cm^2, 1021.312 cm^2) which were on par. Lowest brood area was recorded in bees from L8 (322.013 cm^2).

During April mean brood area declined in all the colonies and black bees from L3 recorded the highest brood area (1066.145 cm^2) which was on par with that of yellow bees from L6, L18 and L11 (998.634 cm^2, 986.938 cm^2, 877.966 cm^2 respectively). The least mean brood area (288.516

Table 3. Monthly variation in brood area (cm^2) of *Apis cerana indica* colonies from different locations during 2012-13

Locations	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Pooled mean
L1	230.115	233.335	395.166	586.083	381.667	493.335	547.195	568. 925	465.122	174.416	200.583	233.083	268.301
L2	268.227	286.667	573.000	713.167	435.583	596.165	675.110	684.251	597.321	302.083	367.916	429.166	424.121
L3	420.250	553.167	912.665	1260.084	879.417	1438.417	1666.667	1587.145	1066.145	693.250	1079.833	1540.750	1094.995
L4	330.557	332.667	516.000	778.000	559.083	676.250	751.312	751.312	452.815	255.916	328.665	393.750	357.786
L5	250.350	253.666	447.667	661.500	470.585	617.333	685.146	668.016	569.417	246.750	278.916	320.583	353.917
L6	345.750	438.833	830.165	1165.000	539.250	742.333	1134.122	1021.312	998.634	527.500	688.749	990.665	801.387
L7	350.125	354.335	601.333	756.835	417.916	496.250	552.101	517.314	418.745	292.335	343.416	373.416	356.978
L8	285.265	343.165	548.835	593.583	291.750	335.250	396.634	322.013	288.516	227.666	222.916	294.083	258.295
L9	380.550	418.000	703.335	827.667	495.667	593.417	675.588	627.158	562.054	335.916	381.500	460.249	434.929
L10	290.785	301.835	508.833	614.000	281.750	367.917	443.772	439.243	333.748	187.582	225.250	262.250	252.207
L11	385.780	429.833	726.167	1035.751	630.085	841.667	1189.309	1098.167	877.966	492.165	789.166	1039.917	799.804
L12	325.256	349.665	557.833	562.000	310.083	382.318	552.787	500.378	387.004	218.995	292.833	356.833	313.916
L13	310.066	348.166	612.830	742.915	466.417	612.835	687.691	622.914	426.545	395.833	445.335	499.416	441.782
L14	365.250	413.500	735.000	825.833	424.250	542.833	659.500	609.275	507.151	368.083	417.833	499.000	448.017
L15	293.355	374.500	588.466	738.917	447.500	454.665	585.111	564.852	498.822	343.666	411.833	490.835	436.289
L16	247.900	348.500	594.500	716.416	388.833	481.000	548.387	519.768	408.111	270.500	309.500	375.166	340.819
L17	374.252	405.333	556.665	701.165	391.583	458.333	532.833	499.637	349.266	235.995	292.000	363.500	310.19
L18	395.275	438.165	729.500	967.832	704.666	979.917	1240.250	1186.867	986.938	719.083	924.085	1105.167	933.818
CD	87.37	94.29	132.681	180.256	227.114	335.183	417.776	301.825	283.365	183.2466	221.6074	264.4436	

cm^2) was recorded in bees from L8. Brood area decreased again during May and highest brood area (719.083 cm^2) was observed in yellow bee colonies from L18 which was on par with that of black bees from L3 (693.250 cm^2) and L6 (527.500 $cm^{2)}$. Common bees from L1 recorded the least brood area (174.416 cm^2). During June, black bees from L3 (1079.833 cm^2) and yellow bees from L18 (924.085 cm^2) recorded highest brood area which were on par. Same trend was noticed during July also with a highest mean brood area of 1540.750 cm^2 in L3 followed by L18 (1105.167 cm^2) followed by L11 (1039.917 cm^2). Least mean brood area (233.083 cm^2) was observed in common brown bees from L1.

c. Pollen storage area (cm^2)

Pollen storage area in different colonies did not show significant variation from August to November and March to July (Table 4). During December, the yellow bees from L11 and L18 showed highest mean pollen storage area (107.749 cm^2 , 103.167 cm^2 respectively) which was on par with those of bees from L16, L13, L14, L17 and the colonies from L8 showed the lowest pollen collection (49.333 cm^2). During January, yellow bees from L11 and L18 had highest mean pollen area (166.000 cm^2, 164.916 cm^2) which was on par with L16, L13, L14 and L15.and bees from L1 showed the least pollen collection (53.749 cm^2). During the month of February the brown bees from L15 showed high mean pollen storage area (114.185 cm^2) which was on par with that of L14, L16, L13, L9, L6 and L18 and the colonies from L7 recorded least pollen storage area (38.873 cm^2).

d. Honey storage area (cm^2)

The results obtained on the honey storage area are presented in Table 5. Mean honey storage area was highest in colonies from L18 and L10 (283.166 cm^2 and 265.500 cm^2 respectively) during September which were on par with that of L13, L11, L9, L6 and L8. All other colonies showed less honey storage area and the bees from L4 recorded the least (115.667 cm^2). Honey storage in yellow bee colonies from L11, black bee colonies from L6, yellow colonies from L18 and brown bees from L8 (377.165 cm^2, 366.165 cm^2, 361.665 cm^2, 361.333 cm^2 respectively) were on par during October followed by bees from location 10, 13, 9, 3,5. The least honey storage (197.333 cm^2) was observed from bee colonies of L16. Highest mean area of honey storage (349.333 cm^2) was recorded in yellow bees from L18 during November, which was on par with that of L6, L11, L3, L9, L4 and L13. Least honey storage was observed in colonies from location 12 (125.333 cm^2) which was significantly low from all other bee colonies. No significant difference observed in honey storage area during December. The highest mean honey storage (326.417 cm^2) was observed in yellow bee colonies from L18 followed by the black bees from L3 (320.583 cm^2). Least mean storage of 133.167 cm^2 was observed in colonies from L12.

There was a gradual increase in honey storage area and it was highest during January to May and then there was a gradual decrease and least mean honey storage was observed during July. Black bees from L3 and L6 had a mean honey area of 446.330 cm^2 and 377.750 cm^2 during January which were on par with that of the yellow bees from location 11 and 18 (416.085 and

Table 4. Monthly variation in pollen storage area (cm^2) of *Apis cerana indica* colonies from different locations during 2012-13

Locations	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Pooled mean
L1	28.148	18.998	22.333	47.617	47.750	53.749	41.612	24.152	21.053	44.249	41.5	33.415	35.381
L2	16.255	47.666	63.667	65.666	78.833	85.750	75.593	62.025	51.422	78.416	82.75	88.583	66.385
L3	85.339	108.833	131.000	77.333	70.833	100.333	70.207	24.158	18.357	67.245	95.917	98.750	79.025
L4	45.174	43.498	44.000	46.916	56.666	75.499	67.083	35.147	28.264	64.665	13.165	20.833	45.075
L5	22.782	52.833	62.833	54.666	57.250	70.332	46.895	36.295	12.379	59.334	62.995	74.000	51.049
L6	55.378	86.000	99.998	62.166	63.583	94.416	92.002	24.337	16.758	53.000	63.245	69.167	65.004
L7	20.991	29.331	42.500	47.667	57.250	57.774	38.873	32.004	41.285	73.917	65.335	59.416	47.195
L8	64.577	62.666	76.000	49.917	40.666	56.916	62.964	48.177	47.276	38.833	35.495	4.584	49.005
L9	32.186	43.666	79.833	64.667	75.915	100.083	99.793	66.117	69.445	44.417	72.335	105.415	71.156
L10	10.228	17.665	16.500	40.500	49.333	68.166	57.573	40.102	31.225	40.667	65.167	85.083	43.517
L11	33.250	39.500	71.333	94.333	107.749	166.000	75.639	31.355	28.007	62.495	105.667	138.083	79.450
L12	12.455	43.500	69.333	44.083	50.916	81.832	45.087	42.147	48.587	26.083	29.165	32.835	43.835
L13	43.202	38.166	42.000	51.916	90.083	132.166	101.754	96.519	96.519	60.083	73.583	102.083	77.339
L14	47.154	60.833	89.667	82.333	89.833	127.083	110.544	85.334	85.334	54.082	61.916	81.082	81.266
L15	39.460	66.500	63.166	46.416	77.416	123.999	114.185	55.284	55.284	60.500	78.665	94.166	72.920
L16	78.266	80.166	74.667	65.667	94.916	134.416	109.232	94.799	94.799	66.749	52.416	81.749	85.653
L17	37.118	31.665	41.500	62.166	80.583	99.334	98.441	67.125	102.125	79.583	83.750	90.583	72.831
L18	80.566	74.000	94.833	98.333	103.167	164.916	84.500	42.366	194.5	53.917	91.915	117.750	100.063
CD	NS	NS	NS	NS	37.3301	44.9681	66.7608	NS	NS	NS	NS	NS	

Table 5.Monthly variation in honey storage area (cm^2) of *Apis cerana indica* colonies from different locations during 2012-13

Locations	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Pooled mean
L1	125.235	164.333	214.665	208.333	143.499	174.833	174.960	181.152	203.551	226.583	150.333	112.000	160.037
L2	148.330	155.835	226.500	233.915	224.500	270.083	301.224	328.344	364.277	282.766	254.417	174.335	228.194
L3	156.245	163.167	279.666	300.417	320.583	446.330	486.706	488.666	489.150	540.083	431.250	351.582	342.834
L4	97.867	115.667	213.500	260.083	227.750	231.583	254.408	262.110	280.647	287.083	229.166	177.000	203.143
L5	135.454	185.666	253.667	247.749	230.835	217.165	233.175	245.355	295.868	266.333	206.915	162.500	206.591
L6	202.536	227.335	366.165	337.335	274.916	377.750	421.934	429.694	432.544	574.916	411.910	312.416	336.573
L7	148.399	187.333	249.500	225.416	265.000	342.916	370.866	378.222	379.312	211.335	181.166	144.167	237.741
L8	197.064	202.167	361.333	216.165	177.165	221.083	239.836	245.890	247.331	123.833	103.165	92.750	187.368
L9	201.470	235.500	315.167	288.166	213.167	285.250	301.679	317.226	381.626	170.916	135.500	119.165	228.756
L10	240.355	265.500	323.666	225.165	192.333	251.583	283.329	288.454	289.251	117.500	86.915	88.583	204.818
L11	197.225	236.335	377.165	323.916	274.000	416.085	449.946	476.997	496.450	214.916	162.666	122.083	289.237
L12	160.777	168.667	203.000	125.333	144.665	165.335	173.892	171.695	179.595	141.999	116.749	96.999	143.131
L13	210.385	250.166	318.166	253.917	258.417	350.417	345.080	355.288	322.297	261.000	179.750	130.495	249.875
L14	145.750	169.000	225.165	231.749	238.834	301.667	283.456	296.224	304.251	215.749	166.245	127.583	209.205
L15	132.258	134.500	219.000	245.500	232.333	273.916	309.253	347.625	354.514	249.165	163.665	126.833	215.658
L16	114.275	152.000	197.333	208.335	213.000	274.583	295.045	297.147	299.225	282.416	189.750	140.665	206.136
L17	165.000	150.500	236.500	240.000	250.665	284.500	296.394	299.256	303.666	169.083	112.916	72.083	199.812
L18	195.274	283.166	361.665	349.333	326.417	496.917	669.339	688.346	687.291	438.333	352.833	256.249	394.089
CD	73.146	85.059	132.009	90.426	NS	205.69	225.74	207.35	217.48	130.878	105.731	83.9197	

496.917 cm^2). Yellow bees from L18 showed the highest mean honey storage area from February to April (669.339, 688.346, 687.291 cm^2 respectively) which were on par with that of black bees from L3. Highest honey storage area (574.916 and 540.083 cm^2) was recorded in black bees from L6 and L3 during May. Comparatively low honey area was observed in colonies with common brown bees than the black and yellow bees.

Table 6. Honey yield (kg) in *Apis cerana indica* colonies collected from different locations of Kerala during honey flow season 2013

Location	Mean honey yield (kg)	Location	Mean honey yield/ colony (kg)
L1	6.416	L10	5.916
L2	11.016	L11	20.642
L3	17.858	L12	7.292
L4	7.833	L13	10.500
L5	6.800	L14	10.408
L6	17.000	L15	7.833
L7	9.958	L16	6.559
L8	6.200	L17	4.708
L9	9.458	L18	18.183

CD 5.6376

Average honey yield (Table 6) was also more in yellow bee colonies from L11 (20.642 kg), L18 (18.183 kg) and black bees from L3 and L6 (17.858 kg and 17.00 kg) and the yield in all were on par. The honey yield in common brown bees ranged from 4.708 kg-11.016 kg only.

e. Disease incidence and absconding behavior

The observations on incidence of disease and absconding/desertion of colonies are presented in Table 7. The results showed that no colonies absconded during August 2012 while during next month (September) one colony from L13 absconded. During October one colony each from L5 and L14 absconded due to disease. In November, 2012 one colony each from L1, L8 and L10 and two colonies from L12 absconded. One colony each from L1, L6, L8 and L10 absconded during December, while the desertion from L6 was not due to disease infection. One colony from L5 and L15 absconded during February and one colony from L12 during March. One colony from L6 absconded in April and it was not due to disease. In May three colonies absconded one each from locations L2, L3 and L16. During next month four colonies absconded one each from L3, L6, L8 and L17. During July one colony absconded from L15. From L3 and L6, three colonies each absconded and none of them had any disease infection, whereas from L5, two colonies absconded due to disease, one during February 2013 and the other during July 2013. Maximum number (5) of colonies absconded during November followed by December and June (4 nos. each) and minimum during March, April and July and September

Table 7. Disease incidence and absconding of *A. cerana indica* colonies (No.) collected from different locations during 2012-13

Locations	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	No. of colonies absconded
L1	-	-	-	D/A (1)	D/A (1)	-	-	-	-	-	-	-	2
L2	-	-	-	-	-	-	-	-	-	D/A (1)	-	-	1
L3	-	-	-	-	-	A (1)	-	-	-	A (1)	A (1)	-	3
L4	-	-	-	-	-	-	-	-	-	-	-	-	0
L5	-	-	D/A (1)	-	-	-	D/A (1)	-	-	-	-	-	2
L6	-	-	-	-	A (1)	-	-	-	A (1)	-	A (1)	-	3
L7	-	-	-	-	-	-	-	-	-	-	-	-	0
L8	-	-	-	D/A (1)	D/A (1)	-	-	-	-	-	D/A (1)	-	3
L9	-	-	-	-	-	-	-	-	-	-	-	-	0
L10	-	-	-	D/A (1)	D/A (1)	-	-	-	-	-	-	-	2
L11	-	-	-	-	-	-	-			-	-	-	0
L12	-	-	-	D/A (2)	-	-	-	D/A (1)		-	-	-	3
L13	-	D/A (1)	-	-	-	-	-	-	-	-	-	-	1
L14	-	-	D/A (1)	-	-	-	-	-	-	-	-	-	1
L15	-	-	-	-	-	-	D/A (1)	-	-	-	-	D/A (1)	2
L16	-	-	-	-	-	-	-	-	-	D/A (1)	-	-	1
L17	-	-	-	-	-	D/A (2)	-	-	-	-	D/A (1)	-	3
L18	-		-	-	-	-	-	-	-	-	-	-	0
Total	0	1	2	5	4	3	2	1	1	3	3	1	

D/A – Diseased and absconded, A – Absconded due to some other reason, ( ) Number of colonies absconded

(one colony each). The results showed that three black bee colonies each from L3 and L6 absconded and none of them had any disease infection and no yellow bee colonies absconded from L11 and L18.

DISCUSSION

The black and yellow morphs of *A. cerana indica* is being reported from Kerala for the first time. Oldroyd *et al*. (2000) reported that *A. cerana* population in Karnataka is composed of two distinct colour morphs: the yellow 'plain' morph and the black 'hill' morph. Later, Banakar (2009) reported that the black 'hill' morph is distributed in Uttara Kannada, Udupi, Dakshina Kannada, Shimoga, Kodagu, parts of Dharwad, Belgaum, Mysore and Chamarajanagar districts. Shruthi *et al.,* 2009 also studied the behavioural traits of two colour morphs from Karnataka. Eventhough, Devanesan (1998) reported four ecotypes of *A. cerana indica,* from Kerala, based on multivariate analysis of 50 morphometric characters, black and yellow morphs were not observed.

The comparative performance on economically important desirable characters of *A. cerana indica* colonies selected from different locations showed that yellow bees and black bees were significantly superior in all the characters including honey production compared to common brown bees.

Maximum bee strength was recorded in black bees and yellow bees compared to common brown bees. This agreed with the report of Shruthi *et al*., (2009) who reported that in Karnataka, bee population in both black and yellow strain colonies of *A. cerana indica* were more and among the strains, black strain colony recorded higher bee population compared to yellow strain colony. Brood area was also higher in both black bees and yellow bees compared to brown bees. Banakar, 2009 reported that brood area in both yellow and black colour morphs was not significantly varying and both colour morphs performed equally exhibiting similar brood growth in Karnataka.

In pollen collection the black and yellow bees do not show significant superiority over the brown bees. It agrees with the report of Banakar, 2009 who revealed that comparative performance of pollen gathering activity in terms of pollen area in colonies of both yellow and black colour morphs exhibited no significant difference. Both the colour morphs performed in similar way in increasing the pollen store area. While Shruthi *et al*., 2009 reported that the black strain showed better performance than yellow strain in pollen storage under Karnataka conditions. This variation may be due to climatic conditions and flora available in the states.

Both yellow and black morphs recorded higher honey storage area than the common brown bees but among the morphs it was high in yellow bees compared to black bees. Similar results were reported by Banakar (2009) from Karnataka. Shruthi *et al*. (2009) reported that black strain and yellow strain of *A. cerana indica* colonies under Shivamogga, Karnataka conditions, both strains stored higher amount of honey and among the strains, black strain recorded more

honey stores compared to yellow strain. These differences observed may be due to variation in genetic character, in bee flora, climatic conditions of different states. In the present study, average honey yield was seen very high in yellow bee and black bee colonies compared to common brown bees.

The black bees and yellow bees had more tolerance/ immunity against diseases compared to common bees. Devanesan (2006) reported that all the ecotypes of *A. cerana* in Kerala are susceptible to TSBV. Amritha *et al*., 2012 reported a new disease incidence from *A. cerana* colonies in Kerala during 2011. No absconding behavior recorded in yellow bees while it was more in black and common bees. The study suggest that the black and yellow morphs with desirable characters can be utilized for selective breeding for production of better performing colonies for commercial beekeeping and enhanced honey production in Kerala.

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Revision of the genus *Cestoplectus* Lamb (Diptera: Chloropidae) with description of a new species from India

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ABSTRACT: A new species of the rare Oriental genus *Cestoplectus* Lamb from India is described. This is the fifth species coming under the genus of which three are distributed in India. A key to all the species of the genus and short notes on Indian species are given.

KEYWORDS: Diptera, Oscinellinae, Botanobiini, *Cestoplectus carinatus sp. n.,* India

INTRODUCTION

Cestoplectus Lamb is a small genus known only from the Oriental Region. Andersson (1977) in his revisionary work on Chloropidae of the world placed the genus along with the genera *Gaurax* Loew, *Gampsocera* Schiner and *Pseudogaurax* Malloch under the *Gaurax* genus group proposed by him. However, Nartshuk (1983, 1987) in her revisionary works on Chloropidae raised the *Gaurax* genus group to the tribe Botanobiini and apart from the above four genera included five more genera namely, *Eugaurax* Malloch, *Hapleginella* Duda, *Leucochaeta* Becker, *Pselaphia* Becker and *Pterogaurax* Duda under the tribe. Of these nine genera only *Gampsocera* Schiner, *Pseudogaurax* Malloch, *Hapleginella* Duda and *Cestoplectus* Lamb are known from India.

Cestoplectus is a small genus earlier known by the type species *C. intuens* Lamb distributed in Sri Lanka, India and Malaysia. In the Catalogue of life 2010, *Cestoplectus brachycephalus* (Frey) and *Cestoplectus hippelatinus* (Frey) described by Frey (1923) from Philippines under *Gaurax* Loew were transferred to *Cestoplectus*. Later Cherian (2011) described one more species, *maculatus* Cherian from India under the genus. He redefined the genus based also on

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maculatus and incorporated characters not given in the original description of Lamb (1918) and in the revisionary work of Andersson (1977).

A new species, *C. carinatus* from Kerala, India is described here, notes on species represented in India are added and a key to all the known species of the world is given. Of the five species, including the new one described here, *brachycephalus and hippelatinus* are distributed only in Philippines, *maculatus* and *carinatus*, sp. n. are endemic to India and *intuens* is found in Sri Lanka, India and Malaysia.

MATERIAL EXAMINED

The type specimen of the new species is retained in the collections of the Department of Zoology, University of Kerala, Trivandrum and shall later be deposited in the National Zoological Collections, Western Ghats Regional Center, Zoological Survey of India. Kozhikode (Calicut).

Morphology nomenclature is followed after Mc Alpine et al. (1981).

RESULTS AND DISCUSSION

Genus *Cestoplectus* Lamb

1918. *Cestoplectus* Lamb. *Ann. Mag. nat. Hist.,* 9(1): 390. Type species: *Cestoplectus intuens* Lamb. By original designation.

Diagnostic characters:

Medium sized partly yellow species with broad head, extremely short and shiny frontal triangle and densely hairy eyes.

Head very broad; frons wider than long or longer than wide, not projecting beyond anterior margin of eye; frontal triangle extremely short, present as a narrow shining area in front of anterior ocellus; face wider than long; facial carina narrow but very rarely broad, reaching from two-thirds to rarely whole length of frons; basal antennal segments usually yellow; *ant* 3 black, reniform to oval, often 2x as wide as long; arista dark, slender, distinctly pubescent; gena less than half as wide as *ant* 3; eyes densely pubescent; head bristles slender but *ovt* and *pvt* bristle like; *orb* about 10, reclinate; *if* not prominent, arranged almost in a row just along outer margin of frontal triangle. Thorax narrower than head; scutum moderately convex, tomentose, with prominent whitish yellow hairs, with or without longitudinal stripes; pleura with or without dark maculae especially on *kepst*; scutellum somewhat rounded, with flattened tomentose disk and hairs as on scutum; 1 *dc* indistinct or weak; *npl* 1+2; *pa* 2 not developed; *as* almost as long as scutellum; *ss* 1-2; haltere orange or infuscated; wing of *Gaurax*-type; discal cell broadened; legs rather long; tibial organ long and prominent, appearing to project a little above tibial surface; abdomen black, densely grey tomentose with whitish yellow hairs; female cerci very slender with fairly dense pale or dark hairs.

Distribution: Oriental Region

As mentioned earlier, this rare Oriental genus is hitherto known by four species, *C. intuens* Lamb, *C. brachycephalus* (Frey), *C. hippelatinus* (Frey) and *C. maculatus* Cherian. A new species *C. carinatus* from Kerala, India is described here.

Key to species of *Cestoplectus* Lamb

1. Scutum reddish to brownish yellow with four dark to black longitudinal bands……..……..2

   - Scutum entirely black or black with yellow tinge at sides and without longitudinal bands……..……..4

2. Eye with short pubescence; antenna entirely black; frons with short, narrow, black fronal triangle; only *ss* 1 present; terminal sectors of R_{4+5} and M_{1+2} almost parallel……..…….*brachycephalus* (Frey)

   Eye with well developed dense pubescence; *ant* 2 yellow; frons with short, narrow, yellow frontal triangle; ss 1 and ss 2 present; terminal sectors of R_{4+5} and M_{1+2} diverging distally…….3

3. Facial carina triangular, not reaching beyond two-thirds length of face; *h* 1 not developed; scutellum brownish yellow; terminal sector of M_{1+2} joining costa near apex of wing…….…… *intuens* Lamb

   Facial carina very broad, reaching epistomal margin; *h* 1 developed; scutellum brownish black; terminal sector of M_{1+2} joins costa beyond apex of wing ……..…*carinatus* sp. n.

4. Frontal triangle not distinctly demarcated; scutum and scutellum shiny; haltere whitish…….....*hippelatinus* (Frey)

   Frontal triangle very small, polished dark brown, distinctly demarcated; scutum and scutellum densely dark grey tomentose; haltere distinctly infuscated in basal half of knob and brownish yellow distally. ……...……*maculatus* Cherian

   (We have not examined the types of *C. brachycephalus* (Frey) and *C. hippelatinus* (Frey). Hence the key is partly based on the original descriptions of the two).

***Cestoplectus intuens* Lamb**

1918. *Cestoplectus intuens* Lamb, *Ann. Mag. nat. Hist; 9* (1): 392. Type locality: Sri Lanka: Peradeniya.

Diagnostic characters:

Head higher than long, very broad, compressed axially; frons wider than long, tomentose, with numerous *fr*; frontal triangle extremely small, shining yellow; face short with triangular carina; ant 2 yellow; ant 3, black, almost 2x as wide as long; arista dark, slender, pubescent; gena narrower than fore tibia; clypeus broad; proboscis and palpi short; head with indistinct *if*, 10 *orb*, short *oc,* well developed pvt and prominent ovt*;* thorax brownish yellow with dark markings; scutum tomentose with four longitudinal dark stripes; other thoracic characters as described under generic characters; wing large, of *Gaurax*-type; haltere yellow; tibial organ large; abdomen tomentose with pale hairs.

Distribution: Sri Lanka: Peradeniya; India: Madras; Malaysia: Malaya.

Remarks: This species was not examined by us and the salient features of the species given are based on the original description of Lamb (1918) supplemented by that of Andersson (1977).

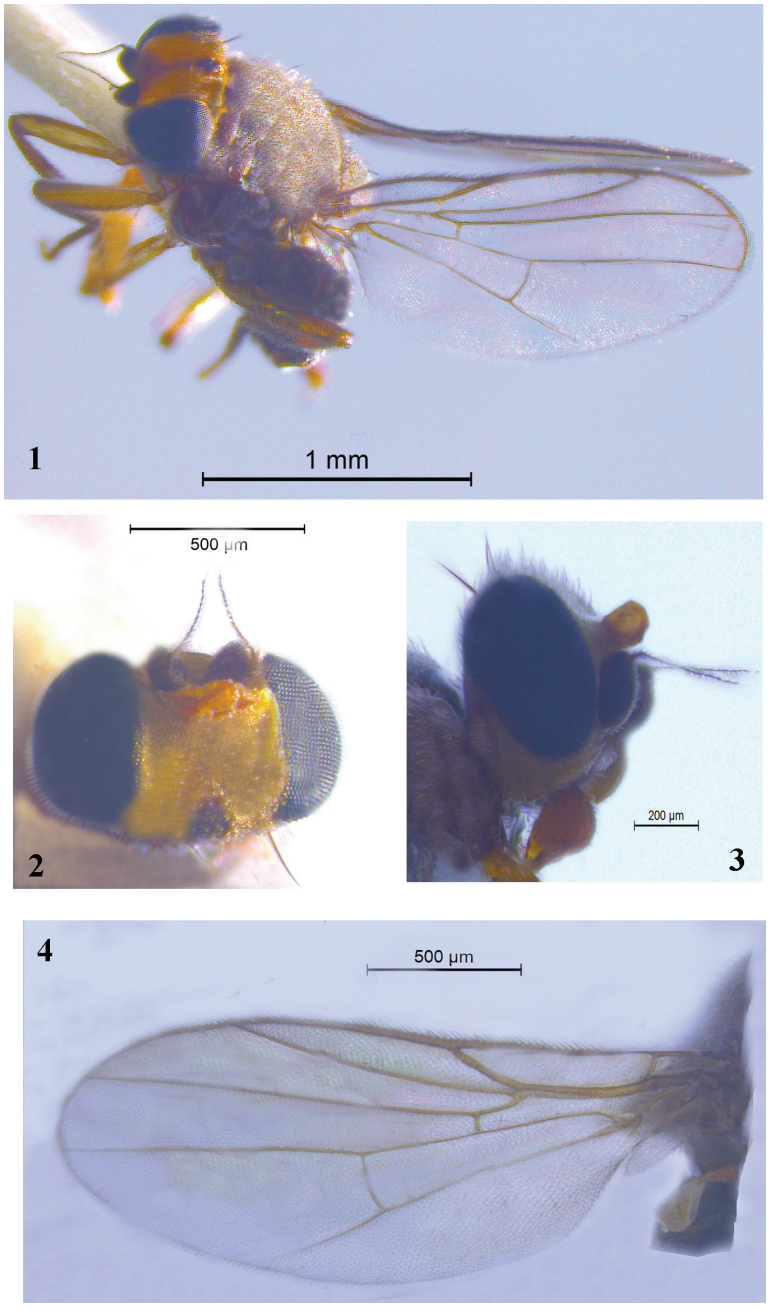
***Cestoplectus maculatus* Cherian**

2011. *Cestoplectus maculatus* Cherian, *Hexapoda,* **18**(1): 1-3. Type locality: India: Kerala:Trivandrum dt., Kariavattom.

Diagnostic characters: Head higher than long with very small frontal triangle; facial carina triangular and running as a low ridge to a little beyond middle of face; antennae widely separated at base and arista slightly thickened at base; gena with well developed oral setae arranged in two rows; postgena narrower than in *intuen*s Lamb; eye suboval with vertical long axis; parafacilaia indistinct; *ivt* slender, unlike in i*ntuens* distinct, about 0.4x the *ovt*; scutum dull black, densely dark tomentose with diffused yellow tinge in notopleural area; dark longitudinal stripes on scutum absent; *kepst* with black macula; scutellum flattened with *as* and only 1 *ss*; wing hyaline with deeply dark brown veins; proportions of costal sectors 2 to 4 in the ratio 18:13:9; terminal sectors of R_{4+5} and M_{1+2} diverging distally; discal cell rather large, conspicuously widening distally; haltere deeply infuscated in basal half and brownish yellow distally; coxae, trochanters, tibiae and femora infuscated; tibial organ well developed; female cerci slender with well developed hairs.

***Length*:** Female 2.86 mm; wing 2.73 mm

Specimen examined: Holotype: Female**:** Kerala: Trivandrum Dist., Kariavattom, 25 m, 8.xii.2005, Coll. A. K. Shinimol.



Plates: *Cestoplectus carinatus* sp. n.:
1. Female fly; 2. Head-dorsal view; 3. Head profile showing bulging ptilinum; 4. Wing

***Remarks*:** *C. maculatus* shows close affinities to *intuens* Lamb but in the former frontal triangle is infuscated, *ivt* is well developed, scutum is predominantly dull black and is without dark longitudinal bands, *kepst* is yellow with black macula and haltere is infuscated in basal half where as in *intuens* frontal triangle is orange, *ivt* is indistinct, scutum is brownish yellow with four dark longitudinal bands*, kepst* is without black macula and haltere is entirely orange.

This species has not been recorded since it was originally published and the male is unknown.

Though in the abstract, keywords, key to species and under remarks the name of the species *C. maculatus* was correctly given in the original description (Cherian, 2011) yet under the caption of description of the species it was wrongly given *maculates. C. maculatus* is the correct name of the species.

***Cestoplectus carinatus*, sp. n.** (Pls. 1-4)

urn:lsid:zoobank.org:act:00E24E6B-43DB-4132-B79C-F4AF759A47A1

Female (Pl. 1), *Head* (Pls. 2-3): Higher than long; frons brownish yellow with numerous pale yellow *fr*; frontal triangle small, deeply brownish yellow, polished and shiny, extending narrowly a little beyond middle of frons; the newly emerged holotype fly is with a bulging ptilinum. Antenna brownish yellow, *ant* 3 oblong arista black, slightly thickened at base. Face deeply concave as in *maculatus,* tomentose, brownish yellow; facial carina well developed, running as a low but broad ridge to epistomal margin unlike in other species of the genus. Antennae widely separated at base; basal antennal segments orange; *ant* 3 wholly deeply black, almost 0.7x as long as wide; arista a little thickened at base; flagellum slender, black with well developed fairly dense concolourous pubescence. Gena about 0.25 x as wide as *ant* 3, dull brownish yellow with well developed oral setae arranged in two rows; vibrissal corner rounded, receding; oral vibrissae short and slender; postgena narrower than in *intuens* Lamb with well developed long pale hairs. Eye oval with vertical long axis; a row of prominent white hairs present along posterior margin of eye and a few more at vertex and in the area below; *ovt* a little longer than the cruciate *pvt*, *ivt* indistinct; rest of the cephalic bristles almost as in *maculatus.*

Thorax: Scutum wider than long, densely grey tomentose, brownish yellow in ground colour with two broad median longitudinal, black bands commencing from the neck region and another two black lateral bands each commencing from behind humeral callus; each of these bands is weakly separated by an yellow streak which almost merge below middle of scutum leaving some remnants of ground colour along its sides; scutellum brownish black, rounded with flattened disc; dorsum of thorax with dense pale yellow hairs; thoracic bristles as in *maculatus* but *ss* 2 though weak is developed unlike in *maculatus*

Wing (Pl. 4): Proportions of costal sectors 2 to 4 in the ratio 6: 4: 2; r-m cross-vein distad of middle of discal cell, opposite 0.57 of its length; terminal sectors of R_{4+5} and M_{1+2} gradually

diverging distally, the latter joining costa beyond apex of wing. Haltere in basal half dorsally weakly infuscated, distally greyish yellow.

Legs: Mostly yellow but most femora and some tibiae and tarsi infuscated as in *maculatus;* tibial organ large and oval.

Abdomen dull brownish black; female cerci slender, with dense hairs.

Length: Female 2.12 mm; wing 1.72 mm.

Holotype: Female: INDIA: Kerala: Alappuzha: Pathiyoor, 25 m, 22-ii-2011, Coll: Jyothi Tilak.

Etymology: The specific name refers to the broad facial carina of the species.

Remarks: *C. carinatus* differs from all known species of the genus in possessing very broad facial carina which reaches epistomal margin. It keys near *intuens* from which it differs in having well developed *h* 1 bristle, brownish black scutellum and in terminal sector of M_{1+2} joining costa beyond apex of wing where as in *intuens h* 1 is not developed, scutellum is brownish yellow and M_{1+2} joins costa at apex of wing.

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ABBREVIATIONS

anepm - anepimeron*; anepst* - anepisternum*; ant 2 - second antennal segment; ant* 3 - third antennal segment; *as* - apical scutellar bristle; 1 *dc* - first dorsocentral bristle; *fr* - frontal hair; h - humeral bristle; *if* - interfrontal bristle; *ivt*- inner vertical bristle; *kepst* - katepisternum; *npl* - notopleural bristle; *oc* - ocellar bristle; *orb* - frontoorbital bristle; *ovt* - outer vertical bristle; *pa* - postalar bristle; *pvt* - postvertical bristle; *ss* - subapical scutellar bristle.

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Effect of different inoculum levels of *Meloidogyne graminicola* Golden and Birchfield on growth and biochemical parameters of rice (*Oryza sativa* L.)

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ABSTRACT: The experiment was carried out to study the effect of *Meloidogyne graminicola* on growth and biochemical parameters of rice, *Oryza sativa* cv. Uma under green house conditions by inoculating with different inoculum levels *i.e*., 0 (uninoculated), 100, 500, 1000, 5000 and 10,000 second stage juveniles per pot. With the increase in inoculum levels of *M. graminicola*, there was a progressive decrease in growth and biochemical characters of the crop. Significant reduction in plant height, fresh weight of plant, dry weight of shoot and root, chlorophyll, protein and starch content of grain at 500 J_2 Beyond this level the damage is stagnating and even at 20 times higher level the plant survives till maturity.

KEYWORDS: *Oryza sativa*, *Meloidogyne graminicola*, rice root knot nematode, chlorophyll, protein, starch

INTRODUCTION

Rice is the world's most important staple food and is cultivated in around 162 mha with an annual global production of 464 mmt (FAOSTAT, 2013). About 53% of the world's rice is grown under irrigated conditions that provide 75% of total global production (Bridge *et al*., 2005). Root knot nematode is an important nematode pest of rice viz. *M. graminicola, M. oryzae, M. javanica*, and *M. arenaria* (Gaur and Pankaj, 2010). Among these species, *M. graminicola* is a primary pest of rice and poses a substantial threat to rice cultivation (Dutta *et al*., 2012). The incidence of root knot nematode, *M. graminicola* in seedling stage and active tillering stages of rice was reported from Kerala (Sheela *et al*., 2005). The changes in growth and biochemical parameters of rice with different inoculum levels of root knot nematode were studied through a pot culture experiment.

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MATERIALS AND METHODS

The experiments were conducted at College of Agriculture, Vellayani during 2014-2015. Each treatment was replicated five times and the pots were arrainged in complete randomized block design (CRD). Uninoculated set of plants served as control. Pure culture of *M. graminicola* was raised from egg masses collected from infested rice roots and multiplied on rice plants maintained in sterilized soil. Subculturing was done periodically to ensure availability of sufficient larval population for inoculation following standard procedures.

Seeds of rice were surface sterilized with 0.1 per cent mercuric chloride and soaked overnight for sprouting. The sprouted seeds were sown in pots filled with steam sterilized soil. Seven days after sowing, seedlings were inoculated with freshly hatched juveniles at the rate of 0, 100, 500, 1000, 5000 and 10,000 J_2 per pot through the holes around the plant within a radius of two centimeters and plugged with the sterilized soil soon after inoculation. To maintain soil moisture in the pot, regular watering was done. Each treatment was replicated five times and the pots were arranged in CRD. Two such lots were setup one lot for 45th day observation and another at maturity.

Forty five days after inoculation, the biometric characters *viz*. plant height, fresh plant weight, dry weight of shoot and root and total chlorophyll content (mg/ g leaf) were estimated from the first set of pots. Protein and starch content of grain (per cent) were estimated from plants in second lot of pots. Chlorophyll was estimated by the method of MacKinney (1941), protein content by Folin- phenol method of Lowry *et al*., (1951) and starch content by Anthron method (Sadasivam and Manickam, 2008). The data were subjected to analysis of variance.

RESULTS AND DISCUSSION

Lowest level of nematode population (100 J_2/ pot) caused significant reduction in plant height (51.46 cm) compared to control (56.52 cm). In next level (500 J_2/ pot) there was further significant reduction (46.80 cm). Though the remaining treatments showed progressive reduction (46.80 to 44.76 cm) in plant height, levels of 1000 and 5000 J_2/ pot came on par with 500 J_2/ pot. 10,000 J2/ pot showed the least and significantly lower plant height (44.76 cm) compared to rest of the treatments (Table 1). Similarly Patil and Gaur (2014) reported that the rice cultivars non-basmati Pusa-44 and basmati Sugandh-5 showed significantly lower plant height with increasing nematode population. Similar results were reported by Abbasi and Hisamuddin (2014) in green gram. They reported that the plant height of green gram inoculated with 800 J_2/ pot and 1600 J_2/ pot decreased by 32.02% and 38.05% significantly over the uninoculated plant. Khan *et al*. (2012) reported that rice grown in nematode infested soil exhibited considerable degree of reduction in plant growth which varied with cultivars.

Lowest level of nematode population (100 J_2/ pot) caused significant reduction in fresh weight of plant (6.53 g) compared to control (7.03 g). In the next level (500 J_2/ pot) there was further significant reduction (5.41 g). Levels of 500 and 1000 J_2/ pot came on par with each other.

Table1: Effect of different inoculum levels of *Meloidogyne graminicola* on growth, chlorophyll content of plants and protein and starch content of grain in *Oryza sativa*

Treatments	Plant height (cm)	Fresh weight of plant(g)	Dry weight (g)		Chlorophyll (mg per g leaf)	Protein content in grain (%)	Starch content in grain(%)
			Shoot	Root			
T1(uninoculated)	56.52	7.03	2.25	1.67	0.93	2.65	86.78
T2 (100 J_2)	51.46	6.53	2.09	1.09	0.79	2.38	65.18
T3 (500 J_2)	46.80	5.41	1.84	0.91	0.74	2.22	68.19
T4 (1000 J_2)	46.10	5.08	1.76	0.84	0.71	2.15	59.83
T5 (5000 J_2)	46.94	4.81	1.73	0.80	0.71	1.62	55.12
T6 (10000 J_2)	44.76	4.53	1.47	0.74	0.68	1.54	50.82
CD (P<0.05)	5.660	0.438	0.118	0.219	0.011	0.246	9.023

Each value is a mean of five replications

Progressive reduction was showed from 1000 to 10,000 J_2/ pot (5.08 to 4.53 g). 10,000 J_2/ pot showed the least and significantly lower fresh weight of plant (4.53 g) compared to rest of the treatments (Table 1). Abbasi and Hisamuddin (2014) reported that in comparison to uninoculated plant, the fresh weight of the whole plant decreased with an increase in nematode inoclum levels. Patil and Gaur (2014) also reported that weight of root and shoot were decreased with increasing nematode population in pot culture experiment.

A progressive decrease in the dry weight of root and shoot were also noted with increase in the inoculum levels of *M. graminicola*. Lowest level of nematode population (100 J_2/ pot) caused significant reduction in dry weight of shoot and root (2.09 g and 1.09 g) compared to uninoculated plants (2.25 g and 1.67 g). The remaining treatments showed progressive reduction (1.84 to 1.47 g and 0.91 to 0.74 g) in dry weight of shoot and root, levels of 500, 1000 J_2/ pot came on par with 5000 J_2/ pot. 10,000 J_2/ pot showed the least and significantly lower dry weight of shoot and root (1.47 g and 0.74 g) compared to rest of the treatments (Table 1). Khan *et al*. (2012) found that the greatest decrease of dry weight of shoot was recorded in the rice cvs. Samba Mahsuri (46.40%) and Sugandh (29.20%). They also reported that the dry weight of root was decreased by 28% (cv. Sugandh) and 23% (cv. R-Dhan). Plowright and Bridge (1990) reported that high nematode population density of *M. graminicola* caused wilting of seedlings along with severe reduction in growth parameters while low population caused only reduction in growth parameters.

In comparison to uninoculated plant, the amount of chlorophyll in the leaves of rice decreased at different inoculum levels. Lowest level of nematode population (100 J_2/ pot) caused significant reduction in chlorophyll content (0.79 mg per g leaf) compared to control (0.93 mg per g leaf). The remaining treatments showed progressive reduction (0.74 to 0.68 mg per g leaf) in chlorophyll content, the levels of 1000 and 5000 J_2/ pot were on par with each other. 10,000

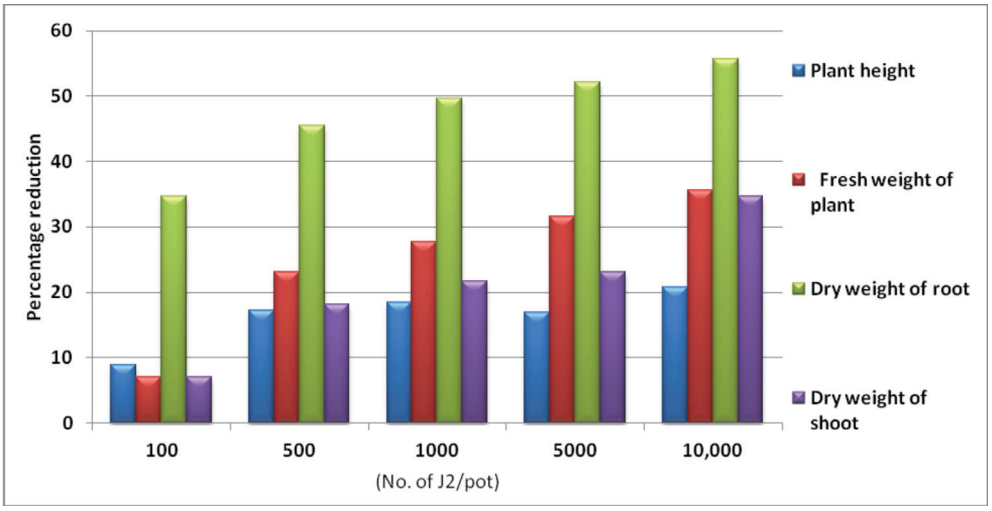

Fig 1. Percentage reduction in Plant growth parameters of rice at different inoculum levels of *M. graminicola* over uninoculated plant

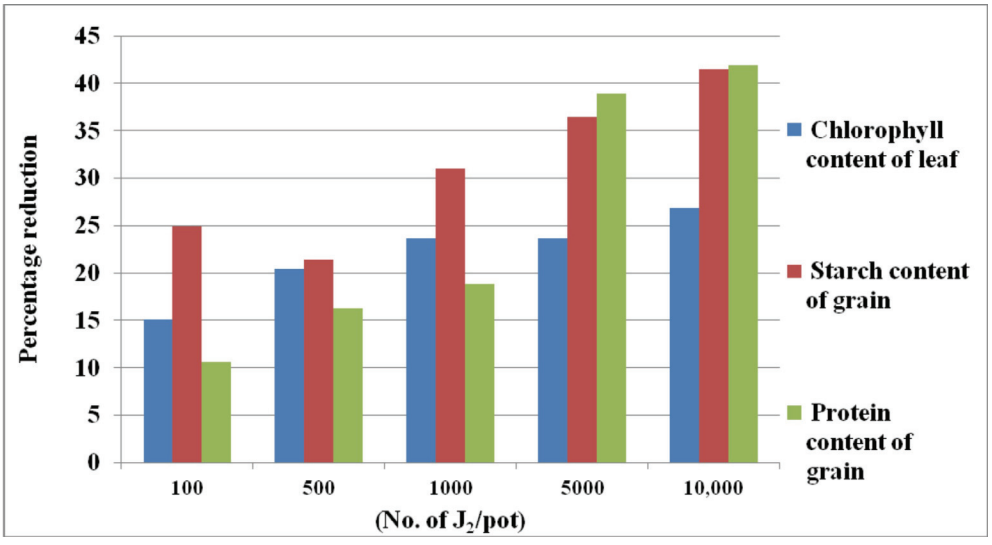

Fig 2. Percentage reduction in chlorophyll content of leaf, starch and protein content of grain at different inoculum levels of *M. graminicola* over uninoculated plant

J2/ pot showed the least and significantly lower chlorophyll content (0.68 mg per g leaf) compared to rest of the treatments (Table 1). There were similar results in tomato, beans, French bean and rice due to root-knot nematode (Lovely and Bird, 1973; Melakeberhan *et al*., 1986; Swain and Prasad, 1988; Ramakrishnan and Rajendran, 1998). Reduction in chlorophyll content of root knot nematode infected plants has been reported by Vashishth *et al*. (1994), Poornima and Vadivelu (1998) and Praveen *et al*. (2006).

Lowest level of nematode population (100 J_2/ pot) caused significant reduction in protein content of grain (2.38%) compared to uninoculated plant (2.65%). Though the remaining treatments showed progressive reduction (2.22 to 1.54 %) in protein content of grain, levels of 100, 500 J_2/ pot came on par with 1000 J_2/ pot. The protein content of grain in plants inoculated with 5000 was on par with that of the protein content of grains in 10, 000 J_2 inoculated plants. 10,000 J_2/ pot showed the least and significantly lower protein content of grain (1.54%). The starch content of grains exhibited reduction at different nematode inoculums levels compared to uninoculated plants. Lowest level of nematode population (100 J_2/ pot) caused significant reduction in starch content of grain (65.18%) compared to control (86.78%). The remaining treatments showed progressive reduction (68.19 to 50.82%) in starch content of grain. Levels of 100, 500 J_2/ pot came on par with 1000 J_2/ pot. 10,000 J_2/ pot showed the least and significantly lower starch content of grain compared to rest of the treatments. Similarly, Abbasi and Hisamuddin (2014) reported that the protein content of green gram reduced at different nematode inoculum levels. Mohanty and Pradhan (1989) reported that the protein contents decreased and amount of free amino acid and amides increased after root knot nematode inoculation in susceptible as well as resistant cultivars of green gram. Patil and Gaur, (2014) reported that the rice grains produced on plants infected with the nematode, *M. graminicola* had poorer nutrient qualities, such as amylase and protein content. Korayem *et al*. (2013) found that crude protein and fat contents decreased in peanut seeds influenced by *M. arenarea*.

The present study revealed that *M. graminicola* affects both growth and biochemical parameters of the plants. Reduction was observed in all the growth characters viz. plant height, fresh weight of plant, dry weight of root and shoot and biochemical characters viz. chlorophyll content of leaf, starch content of grain and protein content of grain with increase in nematode inoculum levels. The highest reduction was observed at the nematode inoculum level of 10,000 J_2/ pot (Fig. 1 and 2). Based on the results of the study, *M. graminicola* was considered as a potential threat to the cultivation of rice. It affected the growth of the rice and quality of the grains. Reduction in chlorophyll content of the leaves reduced the photosynthetic rate of the plant and thus reduced the growth and yield of rice.

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Evaluation of some cooking ingredients decontaminating selected vegetables from pesticide residues

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ABSTRACT: The effect of different safe decontaminating substances on two vegetables (okra and curry leaf) grown in Kerala was studied. The plants were sprayed with a mixture of malathion, chlorpyriphos, quinalphos, profenophos, ethion, cypermethrine, fenvalerate and methyl parathion and samples of okra fruit and curry leaf were collected 1 day and 3 days after spraying. Samples were dipped in different decontaminating emulsion/suspension of tamarind 2%, common salt 2%, turmeric 1%, vinegar 2%, lukewarm water and water for 15 minutes and washed in tap water. Residues of different insecticides in these samples were estimated using gas chromatograph equipped with electron capture detector. Data showed that all treatments reduced the insecticides on the two commodities significantly. The effect varied with the insecticides and the crops involved. The decontamination technology using household substances was identified as a desirable approach for solving the insecticide residue problem in agricultural commodities. Standardization of the technique separately for different commodities and insecticides may be necessary based on a sample survey of the pesticides in different commodities in different locations.

KEY WORDS: Decontamination, pesticide, residue, vegetables

INTRODUCTION

Pesticide residues in food continue to be a matter of concern to humanity. The regulatory authorities in India are focusing on monitoring of pesticide residues in agricultural commodities to ensure food safety for consumers and for better international trade activities. A recent survey of different food commodities sold in different markets of Kerala showed that out of 16,948 samples studied, 290 (1.70%) samples contained residues of various insecticides at levels above those prescribed as safe by FSSAI (AICRP PR, 2012).

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In vegetable samples (141 in number) and fruit samples (56 in number) collected from different locations in Kerala during the period of July to September 2014 under a separate programme of Government of Kerala and Kerala Agricultural University, 12 vegetable samples and five fruit samples had detectable level of pesticide residues. Residues in green chilli, yard long bean, curry leaf, coriander leaf, amaranthus, guava and pomegranate were at higher levels (PAMSTEV, 2014).

This growing concern about pesticide residues in agricultural commodities has demanded immediate government level interventions for solutions. For tackling the problem interventions can be at production, marketing and consumption stages. Most of the vegetables marketed in Kerala are produced outside this state, and hence intervention at production level is almost impossible. The next possibility is intervention at marketing stage *ie*. to collect statutory samples, test them in an accredited testing lab and to take legal action against the food business operator, including seizing of contaminated products. This also remain elusive due to the inadequate infrastructural facilities and complicated legal procedures. Therefore intervention at consumption stage is to be explored as a practical solution for the problem, *ie*. to standardize and popularize decontamination procedures that can be adopted just before cooking/consumption.

Several substances have been reported as effective in decontaminating agricultural products from pesticide residues. They mostly covered earlier pesticides and did not gain popularity and large scale adoption. Recently different decontaminating substances like common salt, vinegar, turmeric etc were tried in India and promising results were also reported (Abou-Arab, A. A. K., 1999, Gardenmo.net, 2011, Varghese and Mathew, 2013 and Vijayasree et al., 2012). These studies do not cover the insecticide residues detected in Kerala in recent surveys and the methodology followed in the treatments also have not been standardized. In this context, two vegetables (okra and curry leaf) seen contaminated with insecticide residues in the surveys were treated with different decontaminating substances commonly used in cooking with a view to assessing the possible levels of decontamination.

MATERIALS AND METHODS

Standardization of pesticide residue estimation

Certified Reference Materials (CRM) of different pesticides having purity ranging from 95.10 to 99.99 per cent were purchased from M/s Sigma Aldrich and stored in a freezer at low temperature, with light and moisture excluded. Solvents used in the study were all glass distilled before use. The analytical method for estimation of residues of pesticides has been validated by conducting recovery studies at three different fortification levels *ie.* LOQ, 5 x LOQ and 10 x LOQ using control samples (samples having pesticide residues below detectable levels).

Decontamination study

To study the effect of different decontaminating materials on removal of pesticide residues, two vegetables *viz.,* okra and curry leaf were selected and the methodology adopted for the decontamination study was as follows.

The okra variety Varsha Upahar was raised in pots under controlled conditions in the Instructional Farm, College of Agriculture, Vellayani. An insecticide emulsion mixture containing malathion (Hilmala 50 EC 2.0 ml L^{-1}), methyl parathion (Folidon 50 EC 2.0 ml L^{-1}), chlorpyriphos (Radar 20 EC 2.0 ml L^{-1}), quinalphos (Ekalux 25 EC 1.60 ml L^{-1}), profenophos (Curacron 50 EC 3.0 ml L^{-1}), ethion (Fosmite 50 EC 3.0 ml L^{-1}), cypermethrin (Cyperkill 25 EC 1.10 ml L^{-1}) and fenvalerate (Fenval 20 EC 0.60 ml L^{-1}) were prepared and sprayed at fruiting stage using a hand sprayer. Okra fruits collected 1st day and 3rd day after spraying were used for pesticide residue estimation.

Curry leaf plants available in the Instructional Farm, College of Agriculture, Vellayani were sprayed with the above mixtures of insecticide emulsions. Curry leaves collected 1st day and 3rd day after spraying were used for residue estimation.

The different decontaminating treatments in this experiment were tamarind 2 % (20g of tamarind pulp extracted in one litre water), common salt 2 % (20g of common salt dissolved in one litre water), turmeric powder 1 % (10g of turmeric powder suspended in one litre water), vinegar 2 % (20 ml of vinegar diluted in one litre water), luke warm water and water (untreated control). Samples of 100 g curry leaves and 250 g okra fruits were dipped individually in the treatments for fifteen minutes followed by washing in tap water. Samples were then cut into small pieces and then homogenized and the representative samples (25 g each) in three replicates were collected, residues in the samples were assessed.

Analytical procedure

The standard QuEChERS protocol was followed to prepare samples and the same was analyzed in a Gas Chromatograph equipped with ^{63}Ni Electron Capture Detector (ECD), fitted with DB-5 capillary column (dimethyl polysiloxane, 30 m x 0.25 mm i.d. x 0.5 µm film thickness)

RESULTS

Recovery study

Multi Residue Methods (MRM) for pesticide residue analysis was adopted for recovery studies. The results demonstrated that the method followed had a satisfactory analytical performance in terms of selectivity and linearity. Good linearity within the range of 0.01-0.5 mg kg^{-1} for the pesticides belonging to OP and SP insecticide groups was obtained. Satisfactory recoveries and RSDs were achieved for most of the pesticides evaluated even at the lowest level of fortification. The mean recovery of all the insecticides used were in the range 70 - 110

per cent and the repeatability of the recovery results, as indicated by the RSD < 20 % confirmed that the method is sufficiently reliable for pesticide residue estimation in curry leaf and okra.

Data relating to the removal of insecticide residues and results of statistical analysis of the data are presented in Table 1 to 8.

Removal of malathion residues:

The performance of different treatments on the residues of malathion on okra fruits and curry leafs is shown in table 1. Turmeric (residue 70.56% < in control) was the best and it was closely followed by tamarind (68.92%) and common salt (62.37%). In samples collected three days after spraying also efficacy of the treatments were in the same sequence, (the percentage reduction ranging from 63.89 to 57.10%). Vinegar, lukewarm water and water were comparatively ineffective, the percentage removal being in the range of 25.88% to 41.77% only. The removal of malathion residues in curry leaf samples harvested at both the intervals was very high in all treatments, the percentage removal being in the range of 69.64 to 95.78%.

Table 1: Extent of removal of malathion residues from okra fruits and curry leaves collected 1st day and 3rd day after spraying subjected to dipping in different treatment solutions for 15 min.

Treatments	Mean per cent removal of insecticides (%)			
	Okra		Curry leaf	
	1st day after spraying	3rd day after spraying	1st day after spraying	3rd day after spraying
2% Tamarind	68.92 ± 1.44 (0.25)	57.10 ± 1.67 (0.13)	91.80 ± 0.24 (0.09)	89.04 ± 0.79 (0.04)
2% Vinegar	38.67 ± 2.83 (0.49)	41.77 ± 1.56 (0.18)	93.24 ± 0.04 (1.02)	76.36 ± 1.53 (0.09)
1% Turmeric	70.56 ± 1.27 (0.24)	63.89 ± 3.75 (0.11)	95.78 ± 0.09 (0.05)	81.34 ± 0.44 (0.07)
2% Common salt	62.37 ± 1.36 (0.30)	62.37 ± 1.36 (0.12)	94.01 ± 1.21 (0.06)	78.84 ± 0.16 (0.08)
Lukewarm water	37.53 ± 1.67 (0.50)	32.53 ± 2.89 (0.21)	87.80 ± 0.92 (0.13)	83.48 ± 0.19 (0.06)
Water	37.53 ± 1.55 (0.50)	25.88 ± 3.31 (0.23)	84.25 ± 0.60 (0.17)	69.64 ± 2.09 (0.10)
Control	(0.80)	(0.31)	(1.10)	(0.36)

Values in parentheses are concentration of insecticides in mg kg^{-1}

The residue of malathion in okra (0.31 mg kg^{-1}) harvested on 3rd day after treatment was only 38.75% of the residue in 1st day sample (0.80 mg kg^{-1}) and corresponding percentage in curry leaf was 32.72%.

Removal of chlorpyriphos residues (Table 2):

On okra samples sprayed with chlorpyriphos and collected one day after spraying tamarind (62.52% removal of residue) was the best closely followed by common salt (54.38%) and

Table 2: Extent of removal of chlorpyriphos residues from okra fruits and curry leaves collected 1st day and 3rd day after spraying subjected to dipping in different treatment solutions for 15 min.

Treatments	Mean per cent removal of insecticides (%)			
	Okra		Curry leaf	
	1st day after spraying	3rd day after spraying	1st day after spraying	3rd day after spraying
2% Tamarind	62.52 ± 3.29 (0.54)	60.22 ± 1.15 (0.20)	66.05 ± 0.93 (1.10)	62.01 ± 2.31 (0.27)
2% Vinegar	50.67 ± 0.70 (0.72)	47.30 ± 1.00 (0.26)	58.61 ± 0.33 (1.34)	34.12 ± 2.60 (0.48)
1% Turmeric	32.14 ± 1.1.9 (0.98)	8.90 ± 1.36 (0.46)	82.93 ± 0.28 (0.55)	47.38 ± 1.45 (0.38)
2% Common salt	54.38 ± 2.136 (0.66)	54.38 ± 2.36 (0.23)	69.36 ± 3.28 (0.99)	38.86 ± 1.17 (0.44)
Lukewarm water	18.68 ± 1.32 (1.18)	17.84 ± 1.89 (0.41)	47.98 ± 3.62 (1.60)	45.72 ± 0.06 (0.39)
Water	9.48 ± 0.40 (1.31)	10.80 ± 0.40 (0.45)	43.59 ± 0.82 (1.83)	34.22 ± 0.81 (0.48)
Control	(1.45)	(0.50)	(3.26)	(0.73)

Values in parentheses are concentration of insecticides in mg kg^{-1}

vinegar (50.67%). Same ranking of the treatments with the percentages of 60.22%, 54.38% and 47.30% on samples collected three days after spraying was observed. In the remaining treatments, the removal of residues ranged from 9.48 to 32.14% only.

In curry leaf harvested one day after spraying turmeric (82.93% residue removal) was the best treatment and it was closely followed by common salt (69.36%) and tamarind (66.05%). In samples collected on the 3rd day after spraying tamarind (62.01%) was the best and it was followed by turmeric (47.38%). Common salt (38.86%) came superior to vinegar and water (34.12 and 34.22% respectively). The residue in okra fruits collected on 3rd day after spraying (0.55 ppm) was only 34.8% of the residue level in samples collected one day after spraying (1.45 ppm).

Removal of quinalphos residues (Table 3):

The results of different treatments on quinalphos residues in okra fruits showed that tamarind, common salt, vinegar and turmeric which removed 65.30%, 63.14%, 54.79% and 52.88% residue from samples collected one day after spraying can be ranked as effective treatments. In the samples collected 3 days after spraying turmeric removed only 36.40% residue while the remaining treatments gave 51.48 to 61.31 per cent residue. All the treatments gave effective decontamination of curry leaf having quinalphos residues the percentages of removal in the 1st day samples being 54.23 to 85.16% and in the 3rd day samples the removal ranged from 50.90 to 69.30% except water which showed the removal of 40.55 per cent only.

Table 3: Extent of removal of quinalphos residues from okra fruits and curry leaves collected 1st day and 3rd day after spraying subjected to dipping in different treatment solutions for 15 min.

Treatments	Mean per cent removal of insecticides (%)			
	Okra		Curry leaf	
	1st day after spraying	3rd day after spraying	1st day after spraying	3rd day after spraying
2% Tamarind	65.30 ± 3.39 (0.42)	61.31 ± 1.09 (0.22)	74.00 ± 1.42 (2.13)	69.30 ± 1.85 (0.60)
2% Vinegar	54.79 ± 3.67 (0.55)	51.48 ± 0.82 (0.28)	70.67 ± 0.23 (1.38)	45.65 ± 3.53 (1.06)
1% Turmeric	52.88 ± 2.88 (0.57)	36.40 ± 1.39 (0.37)	85.16 ± 0.29 (0.79)	57.86 ± 1.03 (0.82)
2% Common salt	63.14 ± 2.10 (0.45)	56.14 ± 2.10 (0.25)	77.35 ± 2.39 (1.20)	50.90 ± 0.37 (2.97)
Luke warm water	39.67 ± 0.78 (0.74)	32.41 ± 0.79 (0.39)	61.46 ± 2.49 (2.05)	58.53 ± 0.58 (0.81)
Water	33.24 ± 2.2.0 (0.81)	18.59 ± 1.22 (0.47)	54.23 ± 0.65 (2.44)	40.55 ± 1.26 (1.96)
Control	(1.22)	(0.58)	(5.34)	(1.96)

Values in parentheses are concentration of insecticides in mg kg^{-1}

The residue level on 3rd day after spraying sample was 47.54% of the level in 1st day sample for okra and corresponding figure for curry leaf was 36.7 per cent only.

Removal of profenophos residues (Table 4):

From samplesof okra collected one day after spraying with profenophos tamarind (73.25% removal), common salt (68.24%) and turmeric (50.60%) could be ranked as good decontaminants. But in samples collected three days after spraying turmeric gave only 41.68% removal while the percentages in the remaining treatments ranged from 52.77 to 65.65 per cent. Lukewarm water and water were relatively less effective.

In the case of curry leaf, samples collected one day after spraying all treatments removed the residues (51.06 to 88.52 per cent) effectively. Samples collected 3 days after spraying also showed good performance (50.95 to 67.52 per cent removal of residues) in all the treatments except common salt and water in which the percentage removal was 48.88 and 44.55% respectively.

Percentage of residues of profenophos in 3rd day samples compared to the 1st day samples was 34.89% in bhindi and 52.84% in curry leaf.

Removal of ethion residues (Table 5):

Tamarind; common salt and vinegar gave 64.03%, 59.73% and 56.06% removal of ethion residue from okra collected one day after spraying while in samples collected on the 3rd day

Table 4: Extent of removal of profenophos residues from okra fruits and curry leaves collected 1st day and 3rd day after spraying subjected to dipping in different treatment solutions for 15 min

Treatments	Mean per cent removal of insecticides (%)			
	Okra		Curry leaf	
	1st day after spraying	3rd day after spraying	1st day after spraying	3rd day after spraying
2% Tamarind	73.25 ± 4.12 (0.80)	65.65 ± 2.48 (0.36)	77.25 ± 0.47 (8.52)	67.52 ± 1.74 (6.43)
2% Vinegar	63.76 ± 3.01 (1.08)	52.77 ± 1.44 (0.49)	68.94 ± 0.18 (11.63)	50.95 ± 2.06 (9.71)
1% Turmeric	50.60 ± 1.26 (1.47)	41.68 ±1.62 (0.61)	87.59 ± 0.09 (4.65)	59.50 ± 0.66 (8.01)
2% Common salt	68.24 ± 2.41 (0.95)	68.24 ± 2.41 (0.33)	88.52 ± 0.98 (4.30)	48.88 ± 1.40 (10.12)
Lukewarm water	27.23 ± 1.67 (2.17)	27.66 ± 2.64 (0.75)	61.46 ± 7.88 (14.40)	59.75 ± 1.78 (7.96)
Water	23.64 ± 2.89 (2.28)	21.66 ± 0.69 (0.81)	51.06 ± 2.41 (18.33)	44.55 ± 0.87 (10.97)
Control	(2.98)	(1.04)	(37.47)	(19.80)

Values in parentheses are concentration of insecticides in mg kg^{-1}

Table 5: Extent of removal of ethion residues from okra fruits and curry leaves collected 1st day and 3rd day after spraying subjected to dipping in different treatment solutions for 15 min.

Treatments	Mean per cent removal of insecticides (%)			
	Okra		Curry leaf	
	1st day after spraying	3rd day after spraying	1st day after spraying	3rd day after spraying
2% Tamarind	64.03 ± 3.94 (1.15)	59.62 ± 0.73 (0.76)	71.26 ± 0.79 (9.02)	67.00 ± 2.11 (6.53)
2% Vinegar	56.06 ± 3.07 (1.41)	52.52 ± 0.68 (0.84)	60.37 ± 0.58 (12.44)	51.71 ± 0.49 (9.55)
1% Turmeric	41.39 ± 1.51 (1.88)	42.38 ± 1.26 (1.08)	86.99 ± 0.31 (4.08)	60.98 ± 0.52 (7.72)
2% Common salt	59.73 ± 1.67 (1.29)	59.73 ± 1.67 (0.76)	70.88 ± 2.77 (9.14)	43.34 ±1.98 (11.21)
Luke warm water	16.96 ± 1.93 (2.66)	11.48 ± 1.35 (1.66)	57.25 ± 1.63 (13.40)	57.15 ± 1.40 (8.48)
Water	5.32 ± 1.58 (3.03)	6.31 ± 2.26 (1.76)	39.94 ± 1.43 (18.85)	43.91 ± 0.59 (11.10)
Control	(3.20)	(1.88)	(31.40)	(19.79)

Values in parentheses are concentration of insecticides in mg kg^{-1}

percentage removal were 59.73%, 59.62% and 52.52% respectively in common salt, tamarind and vinegar. In turmeric the removal was 41.39% and 42.38% removal only on 1st and 3rd day samples. In the case of curry leaf all treatments removed the residues effectively (57.25% to 86.99% removed from 1st day samples and 57.15% to 67.0% removed from 3rd day samples).

Ethion residue in okra harvested on 3 day waiting period was 58% of the residue in samples collected on the 1st day of spraying. The corresponding residue content in curry leaf on 3rd day was 65% of the residue in 1st day samples.

Removal of cypermethrin residues (Table 6):

Removal of cypermethrin was relatively low 54.46% to 57.23% in 1st day samples of okra fruits in treatments with tamarind, common salt and vinegar respectively while the corresponding percentages in 3rd day samples were 52.18%, 45.61% and 50.25% respectively. In the case of curry leaf the removal percentage in treatments except water gave 58.84% to 89.37% removal in 1st day sample and tamarind, vinegar and turmeric gave 53.7% to 59.51% removal in 3rd day samples. In common salt and water treatments the removal of cypermethrin ranged from 36.26% to 49.17% only. The waiting period of 3 days followed in harvest of okra brought down the residue level to 63.6% of the 1st day sample and corresponding reduction in curry leaf was 85.7%.

Table 6: Extent of removal of cypermethrin residues from okra fruits and curry leaves collected 1st day and 3rd day after spraying subjected to dipping in different treatment solutions for 15 min.

Treatments	Mean per cent removal of insecticides (%)			
	Okra		Curry leaf	
	1st day after spraying	3rd day after spraying	1st day after spraying	3rd day after spraying
2% Tamarind	54.46 ± 1.22 (0.05)	52.18 ± 1.16 (0.03)	71.98 ± 0.70 (0.06)	59.51 ± 3.19 (0.07)
2% Vinegar	57.23 ± 1.32 (0.05)	50.25 ± 1.63 (0.03)	66.82 ± 0.49 (0.07)	53.70 ± 3.07 (0.08)
1% Turmeric	32.28 ± 1.16 (0.07)	19.59 ± 1.06 (0.06)	89.37 ± 0.20 (0.02)	56.79 ± 1.68 (0.07)
2% Common salt	54.61 ± 2.09 (0.05)	45.61 ± 2.09 (0.04)	68.14 ± 7.06 (007)	36.26 ± 3.44 (0.11)
Luke warm water	18.17 ± 1.82 (0.09)	15.49 ± 2.90 (0.06)	58.84 ± 12.69 (0.09)	48.63 ± 3.23 (0.09)
Water	6.70 ± 3.04 (0.10)	8.19 ± 2.65 (0.06)	48.58 ± 2.31 (0.10)	49.17 ± 5.18 (0.09)
Control	(0.11)	(0.07)	(0.21)	(0.18)

Values in parentheses are concentration of insecticides in mg kg^{-1}

Removal of fenvalerate residues (Table 7):

Common salt was the best for the removal of fenvalerate residue (73.21%) and it was followed by vinegar (63.67%) and tamarind (56.15%). The corresponding removal in 3rd day sample also was high (49.13% to 73.21%). In curry leaf all the treatments gave high removal in 1st day sample (except water), percentages ranging from 64.95% to 90.25%. In the 3rd day samples satisfactory removal was in tamarind (56.16%) and vinegar (52.69%) only. Residue in 3rd day sample in okra was 61.7% of the 1st day sample and in curry leaf corresponding residue was 66%.

Table 7: Extent of removal of fenvalerate residues from okra fruits and curry leaves collected 1st day and 3rd day after spraying subjected to dipping in different treatment solutions for 15 min.

Treatments	Mean per cent removal of insecticides (%)			
	Okra		Curry leaf	
	1st day after spraying	3rd day after spraying	1st day after spraying	3rd day after spraying
2% Tamarind	56.15 ± 4.98 (0.32)	50.97 ± 1.45 (0.22)	70.39 ± 0.46 (1.06)	56.16 ± 2.72 (1.03)
2% Vinegar	63.67 ± 4.04 (0.27)	49.13 ± 1.84 (0.23)	64.95 ± 0.17 (1.25)	52.69 ± 1.70 (1.12)
1% Turmeric	37.88 ± 2.01 (0.45)	16.97 ± 1.72 (0.37)	87.88 ± 0.16 (0.43)	45.32 ± 10.41 (1.29)
2% Common salt	73.21 ± 2.09 (0.20)	73.21 ± 2.09 (0.37)	90.25 ± 1.88 (0.35)	36.44 ± 1.49 (1.50)
Luke warm water	13.67 ± 0.77 (0.63)	19.73 ± 1.28 (0.36)	84.86 ± 1.76 (0.54)	49.09 ± 2.78 (1.20)
Water	9.70 ± 1.22 (0.66)	11.66 ± 1.83 (0.40)	44.08 ± 2.70 (2.00)	39.90 ± 0.82 (1.42)
Control	(0.73)	(0.45)	(3.59)	(2.37)

Values in parentheses are concentration of insecticides in mg kg^{-1}

Removal of methyl parathion residues (Table 8):

Common salt and tamarind alone were found good for removing methyl parathion residue from 1st day sample of okra fruits while in other treatments residue removal ranged from 11.69% to 40.40% only. In the third day sample the removal in common salt alone was good (54.22%). In the remaining treatments the removal ranged from 11.70% to 37.46%. Regarding curry leaf, all treatments including water gave 50.68% to 74.43% removal in 1st day samples. In 3rd day sample the removal was below 50% in all treatments. The residue levels in 3rd day samples of okra was 43.37% of the 1st day sample and in curry leaf corresponding percentage was 39.84%.

Table 8: Extent of removal of methyl parathion residues from okra fruits and curry leaves collected 1st day and 3rd day after spraying subjected to dipping in different treatment solutions for 15 min.

Treatments	Mean per cent removal of insecticides (%)			
	Okra		Curry leaf	
	1st day after spraying	3rd day after spraying	1st day after spraying	3rd day after spraying
2% Tamarind	51.62 ± 1.483 (0.95)	37.46 ± 1.37 (0.53)	61.22 ± 0.69 (3.30)	43.23 ± 3.17 (1.92)
2% Vinegar	40.40 ± 1.62 (1.17)	31.37 ± 0.82 (0.58)	69.46 ± 0.14 (2.59)	21.77 ± 1.52 (2.65)
1% Turmeric	31.33 ± 0.89 (1.35)	24.71 ±2.29 (0.64)	74.43 ± 0.28 (2.17)	20.70 ± 0.94 (2.68)
2% Common salt	54.22 ± 1.41 (0.90)	54.22 ± 1.40 (0.39)	70.79 ± 3.47 (2.48)	24.02 ± 2.12 (2.57)
Luke warm water	34.43 ± 0.96 (1.29)	15.96 ± 0.78 (0.58)	58.41 ± 3.10 (3.53	31.87 ± 2.66 (2.30)
Water	11.69 ± 1.88 (1.78)	11.70 ± 1.68 (0.71)	50.68 ± 1.49 (4.19)	9.24 ± 1.04 (3.07)
Control	(1.96)	(0.85)	(8.51)	(3.39)

Values in parentheses are concentration of insecticides in mg kg^{-1}

DISCUSSION

The data obtained from the experiments clearly show the effectiveness of the treatments in removing the insecticide residues in okra and curry leaf. From okra removal of malathion residue was comparatively better with tamarind, turmeric and common salt, while vinegar, lukewarm water and water were comparatively inferior. With chlorpyriphos residue, turmeric and water treatments were inferior to the rest of the treatments. Methyl parathion residue was best removed by tamarind and common salt while the remaining treatments were not effective. All the treatments except turmeric and water removed residues of qunalphos, profenophos, ethion, cypermethrine and fenvalerate. In the case of curry leaf the residue levels of insecticides were significantly higher than those of okra fruits. This might be due to lower bulk weight of curry leaf and consequent larger surface area available for holding the residues of insecticide for the same weight of the two commodities taken for the studies. The residues from curry leaf was being effectively removed by all treatments in the experiment including lukewarm water and water. The result bring to light the possibility of reducing the hazards of insecticide residues in vegetables by adopting simple and safe methods of decontamination in post harvest processing before cooking. Because of the variations in the performance with reference to the chemistry of insecticide, crop waiting period etc., standardization of separate techniques for different pesticides and for different commodities (identified through a farm-gate sample survey) may be necessary for tackling the problem.

The rapid fall in residues of different insecticides in samples collected 3 days after spraying when compared to the levels of residues in samples collected 1 day after spraying, highlights

the importance of adopting recommended waiting periods in harvesting the crops after spray operations. This precaution clubbed with the adoption of proper decontamination practices may provide an effective method to solve the hazards of insecticide residues in agricultural commodities. Though the decontaminating effect of a lot of substances have been studied in the recent past the pesticide studied in this experiment and the crops chosen have not been covered in early studies.

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Occurrence of *Conocephalus (Conocephalus) bambusanus* Ingrish (Orthoptera: Tettigoniidae: Conocephalinae) in southern India

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ABSTRACT: *Conocephalus (Conocephalus) bambusanus* Ingrish (Orthoptera: Tettigoniidae: Conocephalinae) is reported from southern India for the first time. It was observed in large numbers in Kodagu District of Karnataka of south India.

KEY WORDS: *Conocephalus (Conocephalus) bambusanus*, First report, southern India

The cosmopolitan genus *Conocephalus* Thunberg, comprising 156 species (Naskrecki and Otte,1999), is recognized by the following characters:vertex more or less laterally flat; apex of vertex round, does not surpass the frontal fastigium, and usually higher than head in lateral view; lateral lobes of pronotum oblique triangular shaped, with a translucent gibbons' area near hind margin above auditory organ; tegmina and hind wings developed or shortened; fore and mid femora usually lack spines on ventral side; hind femora with two spines on knees; fore and mid tibiae without dorsal spurs, with short ventral spurs; tympanum on fore tibiae closed; prosternum with or without two spines; male cercus with inside teeth; ovipositor sword-shaped, brim smooth or with tiny teeth (Zhou *et al.*, 2010). *Conocephalus (Conocephalus) bambusanus* Ingrish (Figs. A and B) can be recognized by the following characters: large body (35-45mm with wings spread); mid femora with ventral spines; hind tibiae with two pairs of ventral apical spurs.

The species is distributed in China, Vietnam, Thailand, Malaysia and Indonesia and so far has been known only from a single female specimen from Darjeeling (Ingrish, 2002).

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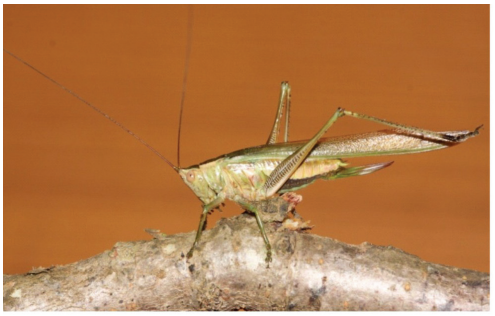

Female **Male**

***Fig. C. Conocephalus bambusanus*Ingrisch - Dead tettigonids**

Occurrence of *Conocephalus (Conocephalus) bambosanus* lngrisch (Orthoptera: Tettigoniidae: Conocephalinae) in large numbers was observed in Kodagu district of Karnataka during July 2010. It was noted in more than 500 hectares in Ponnampet, Gonikoppa, Virajpet, Thithimathi, Mandalpet,Galibeedu, Polibetta, Kotabetta, Chettahalli, Suntikoppa, Kushalnagar and Madikeri areas of the District. They were found resting on banana, rose, *Hibiscus, Duranta, Euphorbia,* curry leaves, bamboo, grasses, china aster, chrysanthemum, Polyalthia, cinnamon, coffee, arecanut, jatropa, lantana, *Erythrina*, and *Ixora*, but no feeding was observed. The grasshoppers were preyed upon by myna, crows and dogs during the day, in the field. The population decreased gradually as most of the hoppers, attracted to light, was found dead (Fig. C).

Specimens of *C. (C.) bambusanus* are deposited in the collections of the Department of Entomology, University of Agricultural Sciences, GKVK, Bangalore.

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T N ANANTHAKRISHNAN

INDIAN ENTOMOLOGIST PASSES AWAY

1925¯2015

Photo courtesy: M. Noble Morrison, CSRTI, Kolar

Taracad Narayanan Ananthakrishnan [TNA], who contributed vastly to Indian biology in general and to Indian entomology in particular, passed away in New Jersey (U.S.A.) at 2.30 PM on Friday, 7 August 2015, leaving behind his wife Menaka, children Ranee and Ramdas, and their children, and us, his graduate students. A little more than 50 of us earned our PhD titles with him, when he was attached to Loyola College, Madras. Well known would be to Entomon readers that he is remembered all over the world for his contributions to Indian insects, especially to the poorly known agricultural and horticultural crop-damaging insects, the Thysanoptera.

Ananthakrishnan's journey with the Thysanoptera commenced in mid-1940s. T. V. Ramakrishna, living in retirement in Taracad in the 1940s advised TNA, then a young academic at Madras Christian College (Madras), to pursue Thysanoptera. M. S. Mani and Y. Ramachandra Rao had their shares of motivation showered on TNA. He first looked at the bionomics of *Arrhenothrips ramakrishnae*, a gall-inducing thrips living on *Mimusops elengi* (Sapotaceae). *Arrhenothrips ramakrishnae* was a natural choice of Ananthakrishnan in getting a grasp of Thysanoptera, because populations of *A. ramakrishnae* were readily and plentifully available in the campus of Loyola College, where he moved in 1948. However, his formal publications on *Rhipiphorothrips cruentatus* and a new species of *Ischyrothrips,* described as *menoni,* preceded his study of *A. ramakrishnae*, which appeared only in the mid-1950s.

In the late 1960s and 1970s, TNA led research in Loyola College passionately. With funding secured from the US PL¯480, he travelled all over India, and shed light on hundreds of Thysanoptera, highlighting their criticality in the context of Indian agriculture, horticulture, and forestry.

Ananthakrishnan unravelled close to 400 new nominal taxa of Thysanoptera, which include 70-odd genera and 300-odd species. While analyzing hundreds of Thysanoptera, Ananthakrishnan was astonished at the phenotypic variations he saw in their populations. He first validated alary polymorphism among thrips in 1959. By the 1970s, he marshalled the concept of insect polymorphism, building on Ernst Mayr's thoughts on organic evolution. Using examples from Indian Thysanoptera, he characterized oedymerous and gynaekoid forms within single populations. The most remarkable element of these findings is that all of these observations and interpretations were made, when we

never knew of the term *Biological Diversity*. He cherished this evolutionary ecological principle until late in his life; he brought out a 200-odd page book *Insect Phenotypic Plasticity* along with Douglas Whitman (Illinois State University, Norman, Illinois) in 2005.

His interest into the ecology and evolution of animals was evolving in the 1960s and the most opportune moment came in early 1970s. His volume *General Animal Ecology* (1976) written with T. R. Viswanathan and published by Macmillan India is a popular textbook for both undergraduate and postgraduate students of biology in India, even today, since it uniquely incorporates ecological data and details of Indian animals.

Pertinent it would be to recall the warm professional association between TNA and K. Karunakaran Nayar of Trivandrum ¯ the brain behind the creation of *Entomon*. The crowning event of Nayar—Ananthakrishnan friendship was the textbook *General and Applied Entomology*, which eventuated with the hard work put in by B. Vasantharaj David and published by Tata¼#McGraw-Hill New Delhi, in 1976.

Ananthakrishnan's interest shifted from the taxonomy of Thysanoptera to the ecology of various insect groups with the appearance of his book *General Animal Ecology*. He used to talk to me at length on the community and population dynamics of insects of different guilds in general and the Thysanoptera in particular. Variations in the guilds of Indian Thysanoptera not only stimulated him intellectually, but also challenged him. Consequently some of us pursued the physiology and ecology of predatory Thysanoptera, some others those of the mycophagous Thysanoptera, and a few others those of the gall-inducing and pollinating Thysanoptera. As a novice, I used marvel at the profundity and proficiency of his knowledge. Much inspiration to Ananthakrishnan in realigning his research interest from taxonomy to ecology of Thysanoptera, at this stage, came from the works of H. G. Andrewartha on the Australian rose-thrips *Thrips imaginis* and *The Ecological Web: More on the Distribution and Abundance of Animals* (1984) by Andrewartha and L. C. Birch.

In the 1960s, he established the Entomology Research Unit, the research wing of the Department of Zoology, in Loyola College. By the late 1970s and early-1980s, Ananthakrishnan gradually tapered his earlier passion for the taxonomy of thrips. Occasionally one or two of us pursued insect taxonomy, while most others — including myself — explored the physiology and ecology of plant-feeding and predatory Thysanoptera and Hemiptera. He directed us to investigate the population dynamics and physiology of feeding by the hemipteroids (the Thysanoptera and Hemiptera in particular), since he was keen to know more about their ecology and evolution than other insects. A clear sense of purpose existed in this thinking. He used to talk to me often that a book on the Indian hemipteroids was badly required, which were gaining greater recognition as insects of economic importance, although, unfortunately, this dream project never materialized until his end. He led his research group with an open mind and encouraged us to explore the bionomics and nutritional physiology of economically important Acridoidea, Coleoptera (Curculionidae), and the Eriophyoidea (Acarina) as well, although most of us studied either the Thysanoptera or the Hemiptera. I vividly remember that at the instance of Vulimiri Ramalingaswamy, then Director-General of Indian Council of Medical Research, 1¯ 2 colleagues of mine pursued the role of urban-house infesting Blattodea in transmitting typhus bacillus. From 1977 to 1980 he directed the Zoological Survey of India ([ZSI], Calcutta), the most exalting position an Indian zoologist could ever dream of. In this role, he realigned the research directions at ZSI, from á¯taxonomical approach to population-ecology based approach in animal systematics. He

inspirationally led the scientific staff of ZSI by urging them to follow contemporary methods in animal systematics, such patterning isozymes, for example.

TNA studied and explained the bionomics of Indian Thysanoptera, from the free-living and polyphagous to the gall-inducing and monophagous species, offering exciting insights into their physiological ecology, especially focussing on their nutritional and reproductive ecology. His investigations on the Thysanoptera that inhabited the weeds along the edges of crop vegetation and how the weeds enabled the movements of pestiferous thrips in and out of the crop ecosystem made vivid impacts in understanding of crop husbandry. His paper on the bionomics of thrips in the *Annual Review of Entomology* in the 1990s is an impressive summary of the state-of-the-art knowledge of the Thysanoptera of the world, and this paper is the jewel in his crown of academic achievement and recognition by the world. He is one of the few in the world, who was invited to contribute articles twice by the Annual Reviews (Palo Alto, California). To me, this is the most extraordinary recognition than many other awards he gained in his lifetime, which I have listed in my article published elsewhere in 2014. In short, he richly and worthily fulfilled the hard dictate of lifting knowledge of Indian Thysanoptera to new heights, which Ramakrishna handed to him in the 1940s.

Having been associated with him for a little more than 25 years, I could go on speaking on his life of perseverance, commitment, and hard work. The most significant message he left for us is that quality science can be done anywhere and with minimal financial investment. This he demonstrated brilliantly by his simple but elegant work done in a 'small' educational institution such as Loyola.

TNA was a brilliant speaker and a fascinating writer. He was a stupendous master, who inspired us at every level of the training he offered us, by his versatility and comprehensive knowledge. His demands were indeed high and we struggled to meet his expectations. However, looking back, I can confidently say that every one of us thinks of him with profound gratitude for the skills and capabilities he has painstakingly embedded in us by awakening the joy of exploration and thus kindling the desire in us to know more.

He was heroic and adventurous. He was attached to a college and not to either a research institution or a university. The limitations were daunting and overwhelming. In spite of the immensity of limitations, he has left *dessins indélébiles* in the pages of Indian and world entomology, by investing hard work with a clear sense of direction and purpose. He craved for excellence in achievement and performance. To us, his students, his life and actions are the inspiring messages. He lived a sophisticated life of academic class and grandeur. In the arena of Indian biology he strode like a colossus.

Verse 6 in the *Advayataraka Upanic*a*t* clarifies the etymology of *guru*: *gu* — shadow, darkness, *ru* — one who quells. Complying with this *Upanic*a*t*-ic clarification, in every imaginable sense, TNA quelled darkness, enabling flawless dazzling light to stream into our heads.

'*Tamaso Ma Jyotir Gamaya:*' — *Brihitaranyaka Upanic*a*t* (Verse 1.2.28).

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I, Dr K. D. Prathapan, Secretary, Association for Advancement of Entomology, here by declare that the particulars given above are true to the best of my knowledge and belief.

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